MALARIA VACCINES AND IMMUNOLOGY

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PLENARY PRESENTATIONS

Malaria Vaccine Status in Africa : Past Experiences, Lessons Learnt and Future Perspectives

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Introduction

Throughout this Conference it has been made abundantly clear that malaria, especially that due to *Plasmodium falciparum,* is Sub-Saharan Africa's bane. It is in this region where malaria is so devastating, causing more than one million deaths each year, and constituting an unbearable burden on the already overstretched health services. Country-specific statistics from tropical Africa almost invariably show malaria as the first or second cause of outpatient attendances, admissions and deaths in health institutions. To add to the misery, the African malaria situation is deteriorating fast. In terms of Disability Adjusted Life Years (DALYs), malaria ranks joint first with respiratory infections, accounting for 10.8 per cent of DALYs in the region. Yet experience from intervention trials in Africa show these figures to be gross underestimates. Economic appraisal would blame a good proportion of Africa's wretchedness on malaria; its direct and indirect costs are put at a staggering US$ 2,000,000,000 annually!

Malaria control in Sub-Saharan Africa is problematic to say the least. Vector control, which ousted malaria from much of Europe and North America, is for the most part impractical in Africa, except for the recently introduced insecticide treated materials (ITMs) which are not themselves devoid of problems. There are already reports of incipient pyrethroid resistance in isolated *Anopheles gambiae* populations. The greatest technical challenge to malaria control in Africa however is the emergence, intensification and spread of anti-malarial drug resistance, sweeping across tropical Africa. As we learned in previous presentations, formerly trusted anti-malarial drugs are in some countries already being abandoned.

The predicament inherent in the above malarial control failures dictate for the search of more effective malaria control tools. Malaria vaccines, in the African context are seen as the promised new tools, mainly because malaria vaccines, like other vaccines in public health use, are likely to be affordable, cost-effective, relatively easy to administer and maintain, acceptable, effective and sustainable in poor-prone African communities. The rest of this presentation will review past African experiences with malaria vaccines, outline the lessons that were learned, and set out future perspectives.

Experience with Malaria Vaccines

The fact that inhabitants of malarious areas, who get frequently bitten by malaria infective mosquitoes, do not always develop clinical malaria led scientists to believe that these individuals develop an effective immune response. Later studies showed that laboratory animals could be effectively immunized with irradiated sporozoites (Nussensweig *et al,* 1967). Soon after, it was shown that humans could be similarly protected (Clyde, 1973).

There were however major drawbacks with immunizations with whole parasites. Over the last decade considerable research has therefore focused on sub-unit malaria vaccines. Such research endeavours have resulted in the identification and isolation of purified antigens, which have been shown to induce strain specific immunity in laboratory animals.

Such achievements are, however, merely preliminary indicators of possible protection in humans. To develop an effective malaria vaccine that is applicable in public health intervention programmes demands considerable clinical and field evaluations beyond laboratory models.

By the late 1980s, there were very promising results from early clinical trials in humans, of recombinant or synthetic malaria vaccine candidates (e.g. Ballou *et al*, 1987, Herrington *et al*, 1987; Patarroyo *et al*, 1987, and Patarroyo *et al*, 1988). Following on from these findings, a number of fields trials were made in several African countries using vaccines carrying components of the circumsporozoite (CS) protein. A meta-analysis for the Cochrane Collaboration was carried out by P. Graves (1997) on field trials in Nigeria, Burkina Faso, Kenya and Thailand, which were random and placebo-controlled, (Reber-Liske *et al*, 1983; Sherwood et al, 1996). Graves concluded that there is no evidence for a reduction in the incidence of malaria by sporozoite vaccines.

New CS based vaccines are meanwhile being developed; some utilizing new adjuvants. In this regard, Stoute *et al* (1997) protected six out of seven naïve volunteers against *Plasmodium falciparum* sporozoite challenge. These promising results were followed, by on going field trials in the Gambia; a preliminary report on initial attempts appeared in the AMVTN Newsletter (1997a). This Conference will learn more on this study. I understand a similar study is underway in Kenya.

Transmission blocking vaccine candidates have been isolated and purified. Some have undergone laboratory testing. The furthest developed vaccine candidate in this category, Pf25 is still undergoing early clinical testing for safety and immunogenicity in the USA and elsewhere. Preparations of field study sites in several African institutions are underway.

Trials of asexual blood stage vaccines have made greater inroads into Africa. Although there are many antigens in this category (e.g. MSP-1, MSP-2, RESA, AMA), only SPf66 has experienced wide-ranging clinical and field testing not only in Latin America, its continent of origin, but also in Africa, and to some extent in Asia (Thailand). SPf66 is a chemically synthesized vaccine against *P.falciparum* , designed and developed by Dr. Manuel Patarroyo in Bogota, Colombia. In preclinical trials, SPf66 induced significant protection against blood challenge in *Aotus* monkeys (Patarroyo *et al,* 1987) and later in humans (Patarroyo *et al,* 1988). In a properly designed field study Valero *et al* (1993) showed a significant reduction in clinical malaria under natural exposure in Colombia. Studies in several Latin American countries obtained similar data, although they were often not well designed.

The Latin American results stimulated intense discussion in scientific circles. A major challenge to SPf66 was thought to be Sub-Saharan Africa, particularly in areas of intense year round transmission. If SPf66 worked under such situations, it would be expected to work elsewhere. Other supplementary, yet crucially important questions were then raised which queried whether SPf66 could lead to undesired immuno-modulation (suppression or pathology) in populations subjected to constant malaria challenge, and whether the protection was peculiar to Latin American *Plasmodium falciparum* strains, as opposed to African strains. The first study on SPf66 undertaken outside Latin America was done in the Kilombero Valley in Tanzania from 1991 to 1994. Results from the initial Tanzania studies involving 586 children 1-5 years of age, showed SPf66 to be safe and immunogenic

(Teuscher *et al*, 1994) and partially effective, giving an estimated efficacy of 31% against first malaria episode. There was however wide 95% confidence intervals (Alonso *et al*, 1994 and Tanner *et al*, 1995). In a follow up study at 18 months after the third dose, the vaccine efficacy was estimated at 25% (95% CI=1-44); there was therefore prolonged protection (Alonso *et al*, 1996) although, again, the confidence interval was still wide.

The above SPf66 study was in young children, although in areas of intense year round malaria transmission, as in the Kilombero valley, the brunt in terms of disease and death is most intense during infancy when much of the malaria is acquired. It was therefore logical to now test SPf66 in infants through the expanded programme of immunization (EPI), with the view of determining its safety, efficacy and interaction alongside standard EPI vaccines. In results just to be published there were no serious adverse effects (Schellenburg *et al*, in press), the vaccine was immunogenic, did not interfere with the EPI vaccines, but only gave an estimated efficacy of only 2% (Acosta et al, in press). The authors therefore concluded that SPf66 in its current alum based formulation, does not appear to have a role in malaria control in Sub-Saharan Africa; they also caution of difficulties in inducing protective immunity against malaria through immunization of infants.

A prelude to the Tanzania study was one *in* the Gambia which, however, differed from the Tanzania study in the age of the study subjects, the period of follow up, and the highly seasonal malaria transmission. The Gambia study showed no statistically significant difference in efficacy, admissions or mortality between the vaccine and placebo group (D'Alessandro *et al* 1995).

In summary, up to now neither of the three malaria vaccine types (sporozoite, sexual or asexual blood-stage) have proved efficacious under field test conditions in Africa. There is therefore need to go back to the drawing boards.

Lessons learnt

Despite failures in producing a highly efficacious malaria vaccine for early deployment in African public health settings, many lessons have been learnt. The last two decades have witnessed considerable progress in the understanding of immune mechanisms that are involved in conferring protection to malaria, the identification of vaccine candidate antigens and their genes, followed by the demonstration of protection in experimental animals.

Early studies demonstrated that humans residing in malaria endemic areas in Africa acquire natural immunity over time. Moreover, experimental vaccination with attenuated sporozoites (e.g. by irradiation) provide effective protection. Unfortunately such an approach would be unwieldy, and would not be technically and economically feasible. Much investment over the last two decades has therefore focused on recombinant or synthetic sub-unit vaccines.

Experience over this period has confirmed that development of malaria vaccines presents formidable difficulties. The encountered complexities relate mainly to the complex malaria parasite biology, human immune responses, pre-clinical, clinical and field vaccine evaluation. It is, for example, becoming abundantly clear that not only do humans possess complex heterogeneities, but parasites and vectors are also just as complex. To this should be added the external and internal milieu. The picture is further complicated by the

complex malaria life cycle, with parasites going through particular developmental stages, each with almost unlimited antigens, only some of which might constitute future vaccine candidates. Then there are likely differences in vaccine batches and their formulations.

At the pre-clinical level there is still lack of good laboratory model systems for human malaria, although the blood stages of the *P.falciparum* can be conveniently cultured.
Similarly, at the clinical level assessment of the efficacy of a candidate vaccine is still problematic. With pre-erythrocytic vaccines one can directly measure parasitaemia after sporozoite challenge, but this is not the case with asexual blood stage vaccines. To me, this constitutes an ethical dilemma. In different areas of endemicity, and in different levels of pre-exposure, there is no level of parasitaemia constituting an agreed end point. Indeed the entire issue of case definition of protection for malaria vaccines in field trials is still unclear, and calls for further elucidation.

Despite considerable research output in basic and developmental malaria vaccine research, the same cannot be said of clinical trials. Of the many antigens developed from basic and pre-clinical research, only a very small proportion ever enter early clinical assessment to say nothing of field testing. To put it rather bluntly progress from the laboratory to the clinic has for the most part been slow if not disappointing. Even where vaccines have entered full field evaluation they seem to reach a dead end, at least with certain formulations, certain age groups, or certain levels of malaria endemicity. To put it mildly “many are called (but only) a few are chosen”.

The current impasse with SPf66 studies, particularly in infants, point at yet more and greater hurdles to be overcome. Success in Phase III testing does not provide a readily available public health tool. Further testing, particularly for compatibility with EPI vaccines and their administration is an absolute necessity.

An examination of the studies reviewed shows that we have indeed come a long way, despite the hurdles. In only a few years there has been great improvement in malaria vaccine field study designs; random, double blind, placebo controlled studies are now the norm, rather than the exception. During this short period, a level of understanding of a critical path and sequence of trials also seems to have been reached.

Future Perspectives

There is no doubt that there is considerable malaria research based on *in vitro* systems and on animal models. Since these do not fall under this presentation, it is assumed that they will continue, and they indeed need to continue and intensify, so as to provide more candidate vaccines.
I would hazard to say that there is pressing need for new malaria research investment focusing on clinical and field research. Such investment should, besides testing malaria vaccine candidates, examine areas that may in one way or another constitute limitations to malaria vaccine development. For example, studies of pathogenesis, or of correlates of protection that will be useful in future efficacy trials.

If future malaria vaccine field trials are to bear fruit that will endure, research capacity in malaria endemic areas should be adequately strengthened so as to reduce the gap between basic research institutions in the north and African field trial institutions. Particular attention should be given to institutions that are likely to participate in malaria vaccine

trials. This aspect will be addressed in another session. At this point I must stress that capacity building should be all inclusive of human resources, infrastructure, supplies, etc.

Human resources development deserves utmost attention. A recent survey (AMVTN 1997b) shows a critical shortage of appropriately qualified African researchers across the board, ranging from the traditional biomedical areas of molecular biology and immunology, all the way to specialties that are crucial in the field evaluation of malaria vaccines and other interventions (e.g. epidemiology, public health, health economics, behavioural sciences and the like). Furthermore, human resources development, must go much beyond training in these traditional areas; training in skills such as good clinical/laboratory practice, study and protocol design, data management and ethics should also be provided. Continuing education of African malaria researchers is essential, given the intellectual isolation they experience.

Meanwhile as new vaccine candidates are being developed, future malaria vaccine testing sites should be better characterized. Detailed information should be gathered on malaria epidemiology, transmission dynamics, pathogenesis, heterogeneity of parasites, vectors and human hosts, spectrum of responses to antimalarial drugs and the like. We shall also need to characterize the type and magnitude of host immune responses in pathogenesis and resistance. These will concern:

- endpoints, surrogate markers, case definition.
- development and validation of field methods for sub-clinical malaria infections.
- establishing repositories of well characterized parasites, vectors, genetic probes, antibody reagents.

There is greater need than ever before to establish working partnerships and networks. In this regard future trials should, whenever possible, be guided by the recently developed WHO guidelines. In order to ensure comparability, multi-centre trials will be desirable, whereby different eco-epidemiological and endemicity settings will be involved. Besides sharing common protocol designs, the participating centres would also share experiences, operational burdens and results. Co-ordination in planning and executing trials is crucially important. Greater involvement of mechanisms as provided by the African Malaria Vaccine Testing Network is highly desirable.

The flow of information on planned or ongoing malaria vaccine trials is at best a mere trickle. In many cases, only research teams know the research plan and its progress. Isn't there need to divulge such aspects as rationale, design and methodology well ahead of the study results?

Conclusions

Although there is ample evidence from field observations and experimental studies that vaccination against malaria is feasible, the development of a safe, efficacious and cost-effective malaria vaccine that can be deployed within the Expanded Programme on Immunization, in an area in Africa experiencing intense perennial malaria transmission, is still evasive. According to this review, SPf66 has failed the rigorous test; RTS.S is still racing ahead, whereas new vaccines including DNA vaccines and new adjuvants are entering the scene. A Luta Continua.

The process involved in pre-clinical, clinical and field-testing is long, demanding and very expensive. The first SPf66 study in Tanzania cost almost US$1 million. It is therefore not likely that a malaria vaccine will be deployed in Africa in less than ten years from now. Given the above realities, malaria control in Africa should continue to rely on available strategies involving chemotherapy or chemoprophylaxis and vector control.

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Basic Research on Malaria Vaccines

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In thinking about standing up in front of the individuals in this audience, many of whom are involved in malaria control, I was struck by the thought that many of you would think, "Here we go again, another talk about malaria vaccines. We've been hearing about these imminent vaccines for fifteen years. There always seem to be fabulous high tech advances, but things don't seem to change very much, and we still don't have a vaccine." The fact is that we don't have a vaccine. However, I hope that all of you will leave this room with some of the enthusiasm and perspective that I have for the tremendous advances that have been made in the past few years in the field of malaria vaccine development, and with some of my confidence that this work is bringing us much closer than we have ever been to fielding an effective malaria vaccine.

To try to put malaria vaccine development in context, I would like to draw upon several of the points that have been raised repeatedly during this meeting. One has to do with the clinical epidemiology of malaria. I believe that 10-15 years ago if I asked any of you who were working on malaria, what the major clinical manifestation of severe disease leading to death in children in the areas with the most intense transmission of malaria was, you would have told me, as I would have told anyone, that it was cerebral malaria. In this meeting, we have heard over and over again, that it is probably severe anaemia not cerebral malaria. In fact cerebral malaria may not be very common in young children in the areas with the most intense transmission of malaria. Likewise, 10-15 years ago, if asked what age group of children contributed most to the mortality of malaria, I think it would have been unlikely for anyone to have said infants. The common wisdom was that infants were protected by maternally transferred immunity. However, it is now estimated that in some areas with intense malaria transmission, 25%-50% of malaria-related deaths occur in children less than 8 months old.

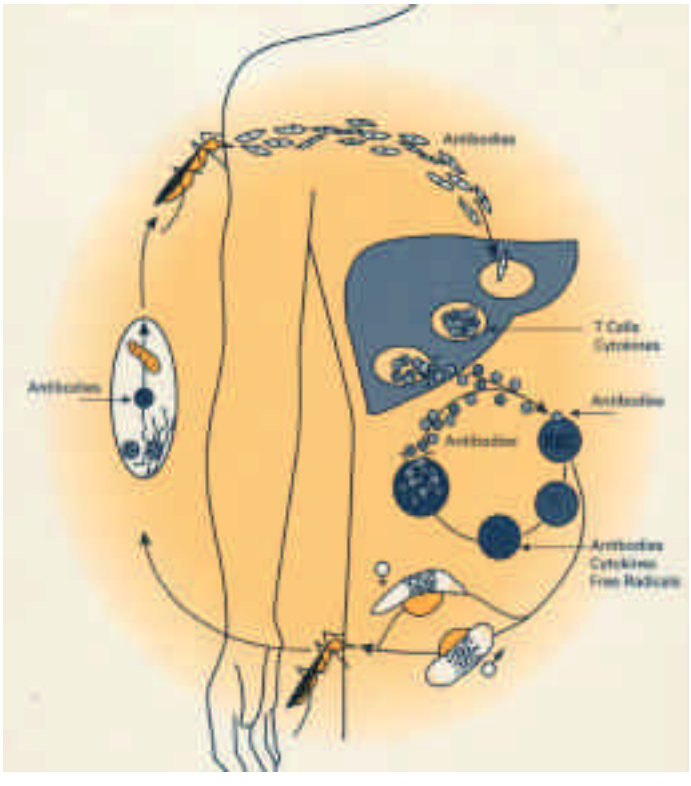


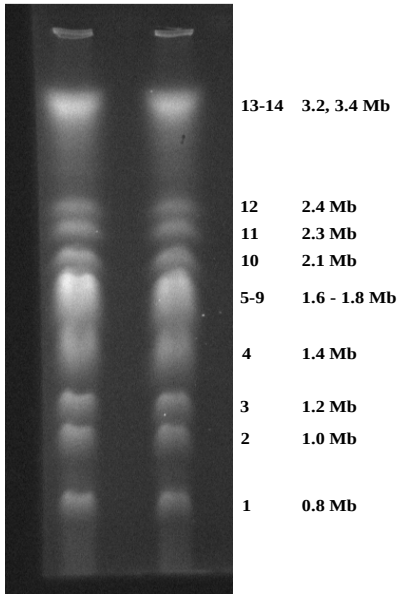
Figure 1: The life-cycle of the malaria parasite

Lastly, how many molecular biologists are in the audience? How many of you would have believed me if I told you two years ago that in the *Plasmodium* falciparum genome there is probably a family of genes encoding variant surface proteins with an estimated 2-2.5 times more copies than Var genes? I think that no one would have believed me. However, in one

fell swoop the genomic sequence data from chromosome 2 of *P. falciparum* has raised that possibility. My point is that if one considers how incomplete our understanding of the clinical epidemiology, immunology and molecular biology of *P. falciparum* was 5-10 years ago, it is not surprising that we have not made the rapid progress in vaccine development that we had hoped for. Specifically, why isn't there a malaria vaccine after so many years? One reason is that we are faced with a formidable foe. We have a complex parasite, which has a multi-stage life cycle, and stage-specific expression of proteins. That means that if a protein on the surface of sporozoites is a major target for antibodies, even if we elicit high levels of anti-sporozoite antibodies, those antibodies will generally not recognize the blood-stage of the parasite's life cycle!

Furthermore, *P. falciparum* has a large genome of 30 megabases on 14 chromosomes (Figure 2) and an estimated 6,000 genes. And the parasite has allelic and antigenic variation. In regard to allelic variation, we know that a single individual can be infected with more than five different strains of *P. falciparum* .

Figure 2: Fourteen chromosomes of *P. falciparum*



Another reason why it has been difficult to develop vaccines is that the human response to the parasite is quite complex. This response is in large part a reflection of the human host's genetics, the transmission dynamics of the parasite, and perhaps even the age of the host. We all know that individuals with sickle cell trait generally do not develop severe malaria. Recently it has been suggested that certain genetic characteristics make one more susceptible to severe disease. However, the fact is that we may actually know very little about the relationship between host genetics and the response to infection. I am hopeful that the elucidation of sequence of the human genome and the development of scientific tools to use these data will lead to a much better understanding of the role of host factors in determining the severity of disease associated with infection. Immune responses are also dependent on transmission dynamics. In areas where transmission of *P. falciparum* is most intense, infants are at highest risk of developing severe and fatal malaria. In areas with less intense transmission, older children have a higher incidence of severe and fatal disease than do infants. The age of the individual may also be important. A number of reports have suggested that among non-immune children and non-immune adults, the adults are actually more susceptible to developing severe disease after their first infection than are children. However, the adults develop acquired immunity faster than do the children. We have much to learn about the impact of host genetics, transmission dynamics, and age on the pathogenesis and clinical manifestations of malaria.

There are currently three general approaches to malaria vaccine development being pursued. The most work has been done and progress achieved on an approach focused on maximizing the magnitude and quality of immune responses to a single or a few key antigens, such as the circumsporozoite protein (CSP) and merozoite surface protein 1 (MSP1), by immunizing with synthetic peptides or recombinant proteins in an adjuvant. These vaccines are being designed to primarily induce antibody and $CD4^+$ T cell responses, but there is also interest in eliciting $CD8^+$ T cell responses. The second

approach is to induce good or optimum immune responses against all of the approximately 15-20 identified potential targets proteins by immunizing with DNA vaccines and boosting with either DNA vaccines, recombinant viruses or bacteria, or recombinant proteins in adjuvant. The goal is to elicit antibody, $CD4^+$ T cell and $CD8^+$ T cell responses. The third approach is to try to duplicate the whole organism immunity induced by immunization with radiation attenuated sporozoites and natural exposure to malaria. Success in this area will be dependent on the sequencing of the *P. falciparum* genome and developing methods for exploiting this genomic sequence data. It remains to be established how such vaccines will be constructed.

Many malariologists believe there may not be only one malaria vaccine. I am not certain I'm amongst them, however, I find it very useful to think about the extremes of requirements for a malaria vaccine. One requirement is to reduce malaria associated mortality and the incidence of severe malaria in infants and children in Africa. There has been considerable discussion at this meeting regarding how to do this. The other extreme requirement is to prevent all clinical manifestations of malaria in individuals from areas with no malaria who travel to areas with malaria.

Children living in Navrongo in northern Ghana are frequently infected with *P. falciparum* , and when infected develop a febrile illness that prevents them from playing or going to school. However, they rarely, if ever, develop severe disease or die of malaria. Essentially all the deaths in this region occur in the first 1-2 years of life. In Navrongo there is a single hospital that serves the approximately 175,000 residents of Navrongo as well as residents of neighboring areas. In 1996, 41% of deaths in the hospital were attributed to malaria, and another 18% to anaemia. Since much of the anaemia can be attributed to malaria, this suggests that 50% of all deaths in the hospital were caused by malaria. We would like to have a malaria vaccine that, from an immunological point of view, turns 6 months olds into the 4 or 5 year olds in the picture. In other words, a vaccine that prevents death without necessarily preventing infection or even mild illness.
Saradidi is a place in western Kenya, where the transmission intensity of malaria is similar in many respects to the transmission intensity in northern Ghana. It is not infrequent for someone born in western Kenya to attend university in Nairobi, and then get a job, get married, raise a family, and settle in Nairobi where there is no malaria transmission. The children of these Nairobi residents are non-immune to malaria. When they visit their families in western Kenya on school holidays they are at high risk of contracting malaria and rapidly developing severe disease. There is very little mention in the malaria literature of the increasing numbers of non-immunes living in countries with endemic malaria who must receive short-term protection against malaria by a vaccine. Because of their susceptibility to rapidly developing severe disease, because they will not have the repeated exposure that could lead to boosting of vaccine-induced immunity, and because they are only visitors, I would think that their parents would want them to have a vaccine with the same preventative profile as a vaccine required by travelers from North America or Europe. What would you choose in that setting?

So, in thinking about developing a vaccine, and the target population for a vaccine, we need to think about the patterns of morbidity and mortality. We desperately need much more sophisticated information regarding the epidemiology of severe malaria and malaria associated mortality. I hope that many of you will return home, and begin to systematically collect the epidemiological data that will be critical to designing malaria vaccine trials. If the majority of deaths from malaria in an area are in 6-12 month old infants, we would be foolish to do a vaccine trial in 2-4 year olds. Likewise, if the majority of deaths are in 2-4

year olds, data on vaccine efficacy will be acquired most rapidly if we vaccinate 1-2 year olds, not newborn infants. Furthermore, if certain groups in the population almost never die of malaria because of the genes that they have inherited, then it makes no sense to include them in a vaccine trial aimed at determining whether a vaccine reduces mortality.

We think that in northern Ghana it is infants who are primarily dying of malaria and we think that in the Gambia it may be 2-5 year olds who are primarily dying. Why do they die? It appears that in northern Ghana severe anaemia caused by malaria may be the major cause of death and in the Gambia, cerebral malaria. Is it possible that we may need different vaccines for infants who die of severe anaemia and for older children who die of cerebral malaria? I certainly do not know the answer to that question, but I do know that I need to understand the epidemiology of malaria in the area if I am going to adequately design a vaccine trial to try to reduce the major cause of death in that area. Data from the large studies with insecticide impregnated bednets could be very helpful in this regard. Those studies showed 18% to I believe approximately 50% reduction of all-cause mortality. Who were the individuals whose lives were saved? My recollection from reading some of these studies is that the reduction in risk was not significantly different in infants or children up to the age of five or so. However, since the infant (1-11 months) and even 12-23 month old mortality rate was up to 4 times higher than the mortality rate in older children, the numbers of deaths saved in the younger age groups was significantly higher than in the older age groups. A more detailed analysis of these data may provide information that could be enormously helpful in optimally designing malaria vaccine trials.

We actually have human models for the two extremes of vaccines I mentioned earlier. In regard to a vaccine to prevent death and severe disease, we know that naturally acquired immunity is the model. If you make it past a certain age in the areas where malaria is transmitted, you will become reinfected and you will become clinically ill, but you will not develop severe disease or die. In regard to a vaccine to prevent all manifestations of malaria, we have immunization with radiation-attenuated sporozoites. Exposure of humans to the bite of greater than 1000 irradiated mosquitoes carrying *P. falciparum* sporozoites in their salivary glands over 4-6 months protects virtually all recipients against exposure to 5 infected mosquitoes 2 weeks after the last dose of irradiated sporozoites. The protection is not strain-specific and lasts for at least 9 months.

The data included in this figure (Figure 3) were generated in 1986 in Saradidi in western Kenya. Almost identical data have been generated recently in a study in Navrongo in northern Ghana. When adults who have lived their entire lives in areas with intense transmission of malaria are radically cured of malaria, virtually all of them become reinfected within 4-6 months. Naturally acquired immunity is not a model for the vaccine to prevent all clinical manifestations of malaria in travelers, because naturally acquired immunity does not prevent the development of blood stage parasitaemia. In fact, in the recent study in northern Ghana, at the time of identification of parasitaemia approximately 30% of the adults were symptomatic. However, the rate of developing recurrent infections and the parasite densities of recurrent infections are much lower in the adults than in their young children, and none of the adults develop severe disease. However, this data clearly demonstrate that naturally acquired immunity is not a human model for a vaccine for travelers designed to completely prevent blood stage parasitaemia and the clinical manifestations disease.

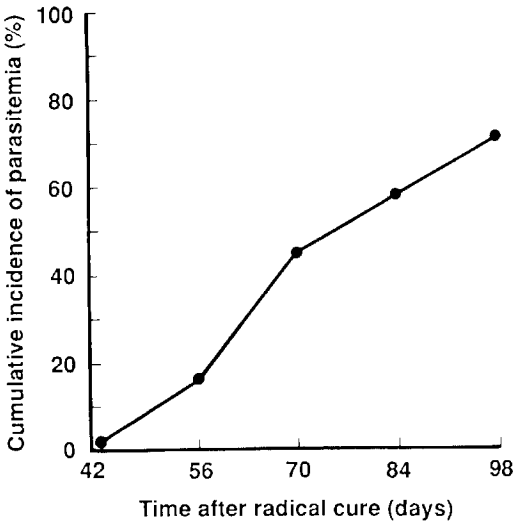


Figure 3: Rate of *P. falciparum* reinfection in Saradidi

The "modern" approach to vaccine development is to identify the mechanisms of protective immunity in the human model system; to identify the antigenic targets of the protective immunity in the model, and then to develop a vaccine delivery system that induces the required immune responses against the identified targets. There are very few vaccines that have been developed this way, and none against any infectious agent as complex *as Plasmodium falciparum* .

What do we know about immunizing with the irradiated sporozoites? First of all, essentially everyone who is immunized properly is protected. This means there is no genetic restriction of protection. The protection is not strain-specific. Individuals immunized with parasites from Africa and challenged with parasites from South America are protected. Protection lasts for at least nine months. The irradiated sporozoite vaccine would be an ideal vaccine for travelers. However, it is totally impractical to conceive of immunizing hundreds of thousands of people by the bites of thousand of infected mosquitoes. Thus, there has been considerable work over the last 30 years to understand irradiated sporozoite-induced protection, and develop a subunit vaccine that duplicates this excellent immunity. We believe that the primary protective immune mechanism in the irradiated sporozoite model involves $CD8^+$ T cell recognition of parasite-infected hepatocytes. However, antibodies and $CD4^+$ T cells almost certainly also play a role in the protection. The targets of the $CD8^+$ T (and $CD4^+$ T cells) are parasite proteins expressed by irradiated sporozoites within hepatocytes. However, sporozoite surface proteins are also the target of inhibitory antibodies.

With pre-erythrocytic stage vaccines we are trying to prevent sporozoites from entering hepatocytes, or developing within hepatoctyes. The irradiated sporozoite vaccine does not elicit immune responses against the major merozoite surface proteins. However, strictly speaking, a pre-erythrocytic stage vaccine could also be designed to elicit antibodies that recognized proteins on the surface of merozoites released from hepatocytes, and thereby eliminate the parasites that may have made it through the anti-sporozoite, and anti-liver stage blockage described above. If parasites actually do invade erythrocytes and begin the process of development, I believe that they will cause disease.

There are quite a few human studies planned or in progress for pre-erythrocytic *P. falciparum* vaccines. We heard extensively yesterday about RTS,S which has been developed by SK BIO (Smith Kline Biologicals) in Belgium, in collaboration with the Walter Reed Army Institute of Research (Stoute *et al.* 1997). We also heard about studies in progress or planned in which RTS,S is being combined with TRAP (also known as PfSSP2). There is a branched chain multiple antigenic synthetic peptide vaccine based on the repeat region of the *P. falciparum* circumsporozoite protein (PfCSP) which has been developed by New York University and the University of Geneva and is being tested in clinical trials at the University of Maryland. There is also a carboxy-terminal synthetic peptide from the PfCSP developed at the University of Lausanne which is in Phase I clinical trials now. At the Naval Medical Research Center (NMRC), we have conducted a

Phase I safety and immunogenicity clinical trial of a PfCSP DNA vaccine, and are planning another trial next month. Next November, we plan to initiate a trial of a 5 gene pre-erythrocytic stage DNA vaccine that includes genes encoding 5 proteins expressed by irradiated sporozoites in hepatocytes. This project is called MuStDO 5.1 (Multi-Stage Malaria DNA Vaccine Operation-5 gene, iteration 1). This is collaboration between NMRC, Vical Inc., the United States Agency for International Development, the Institute Pasteur (Paris), and Pasteur Merieux Connaught. At Oxford, they are going forward in clinical trials with a *P. falciparum* pre-erythrocytic stage multi-epitope vaccine; recipients will receive the first dose as a DNA vaccine, and the second dose as a recombinant attenuated vaccinia virus (MVA) expressing the same epitopes.

There is considerable hope for these pre-erythrocytic vaccine approaches, certainly for the second indication I described, which is preventing all manifestations of the disease. However, there has been quite vigorous debate as to whether a pre-erythrocytic stage vaccine on its own would reduce mortality in children in Africa. If such a vaccine were perfect, it certainly would be effective in this regard, because there would not be any parasites escaping from the liver into the blood stream. If it were less than perfect, most scientists believe that there would have to be substantial anti-asexual erythrocytic stage immunity in recipients. The fact of the matter is that most of the individuals, including infants, that we contemplate immunizing will have some degree of anti-asexual stage immunity. A question that has been raised for which there is no answer is, "What will happen if such a vaccine is perfect or almost perfect for a year or more, and then rapidly becomes ineffective?" Will overall malaria morbidity and mortality worsen since recipients would not have developed anti-erythrocytic stage immunity? We know that the clinical presentation of disease varies in relation to transmission intensity. Others (Snow *et al.* 1997) have wondered if by changing the host-parasite dynamic interactions will we alter the pathogenesis of disease, and in some cases make things worse? There are no answers to these questions, and prospective studies will have to be designed to address them. However, I am encouraged by the fact that preliminary reports from long term studies of insecticide impregnated bed net studies are not finding any delayed increase in morbidity or mortality. One could even characterize insecticide impregnated bednets as being analogous to "leaky" pre-erythrocytic stage vaccines.

The other human model for vaccine development is naturally acquired immunity. In areas with annual, stable transmission, there is little to no severe disease or malaria associated deaths after the age of 7-10 years. In areas with the most intense transmission, the transition to this immunity against severe malaria occurs even earlier, perhaps during the second year of life. Even adults become infected and develop symptoms attributed to malaria, but the incidence of new infection, and the density of parasitaemias decreases with age. Most malariologists believe that antibodies against parasite proteins expressed on the surface of infected erythrocytes and merozoites and in apical organelles play a central role in this naturally acquired disease modulating immunity (Figure 1). However, biologically active molecules including cytokines, nitric oxide, and free oxygen intermediates, either released from $CD4^+$ T cells after an antigen-specific interaction, or released from reticulo-endothelial or other cells after non-specific activation also probably contribute to this immunity. Furthermore, the pathogenesis of the disease itself may be mediated by these same host-derived biologically active molecules, perhaps elicited by putative toxins released from the infected erythrocytes. Antibodies against these toxins may contribute to naturally acquired immunity. Finally, it seems intuitive that immune responses against

sporozoites or infected hepatoctyes that limit the numbers of parasites that emerge from the liver into the bloodstream must also play a significant role.

An important question for scientists studying naturally acquired immunity is, "How rapidly does naturally acquired immunity to mortality actually develop?" In *Aotus* monkeys, a non-natural host for *P. falciparum* , the first exposure to infected erythrocytes of the FVO strain of *P. falciparum* is almost always fatal. However, most survive the second FVO challenge, and all will survive the third FVO challenge. When patients with neurosyphilis were treated by infection with *P. falciparum* a similar pattern was reported. Recently, there was a report suggesting that in areas with intense transmission of *P. falciparum* , this anti-mortality immunity may develop after only one or two exposures to *P. falciparum* (Gupta *et al.* 1999*)*. I think that data derived from studies further exploring this question will be critical to developing and studying effective malaria vaccines. The data may vary considerably depending on the transmission dynamics and epidemiology of the disease, but we will never know until appropriate field studies are executed.

I mentioned that *Aotus* monkeys re-challenged with the FVO strain of *P. falciparum* rapidly develop anti-parasite immunity. I would like to tell you about a study that was recently completed by Dr Trevor Jones from NMRC, and Dr Nicanor Obaldia from Promed Inc. at the Gorgas Memorial Laboratory in Panama. They exposed *Aotus* monkeys (*Aotus lemurinus lemurinus*) 8 times to *P. falciparum* infected erythrocytes. After the first infection with 10,000 FVO-infected erythrocytes parasites, 8 of the 8 monkeys became infected, the pre-patent period was 8.2 days, the maximum parasite density was 840,000 parasites/µl, the geometric mean density being 443 parasites/µl and all of the monkeys had to be treated or they would have died. With their second exposure, 8 of 8 become infected, the pre-patent period lengthened to 12 days, the maximum parasitaemia was reduced by approximately 50%, the mean peak parasitaemia was reduced by approximately 75% to 107,000 parasites/µl and only 5 of the 8 had to be treated. With their third exposure, only 6 of the 8 developed detectable parasitaemia, the pre-patent period was 19 days, the peak was 31,000 parasites/µl, the geometric mean was 220 parasites/µl and none of the 8 had to be treated. With their 6th and 7th infections, none of the monkeys developed parasitaemia; they actually had sterile protective immunity against the blood stage of *P. falciparum* . These monkeys with sterile protective immunity were then challenged with erythrocytes infected with the CAMP strain of *P. falciparum* . Six of the 8 became infected, the pre-patent period which had been 30 days after the fifth FVO challenge went back down to approximately 8 days, the peak parasite density was 11,000/µl and the mean was 1,400 parasites/µl and none of the 8 had to be treated. They did not have sterile protection against parasitaemia but were protected against death. However, 2 of the 8 monkeys developed parasite density levels that would have made humans quite ill (approximately 10,000 parasites/µl) and certainly would have caused fever, and these 2 monkeys and a third monkey had a drop in their hematocrits (packed cell volumes) of greater than 50%. I believe that these results are quite instructive. If we are developing a vaccine to reduce mortality, but our outcome variables in early field trials are parasite densities greater than 5,000/µl, fever, or development of anaemia, we may find that a vaccine that would be effective in reducing mortality was discarded before it was tested for this indication.

Thus, it is critical to consider what outcome variables to measure in field trials of vaccines, and what populations to study. A primary goal is to reduce mortality and severe disease. The problem is that initial studies may not measure these outcome variables, and there is the potential for entirely missing (discarding) a vaccine because we did not measure the proper outcome variable(s). It will be difficult to use severe disease and death as the

primary outcome variables in initial studies, because this would require very large sample sizes. Acquiring data that will allow us to reduce sample sizes by focusing only on groups at highest risk will be enormously important in the future. Some groups are working on identifying surrogates of severe disease and death, parasitological, hematological, biochemical, or clinical manifestations that are predictive of severe outcome. This will not be easy and may ultimately be unrewarding if the surrogate markers occur only in those who will develop severe outcomes as this would not allow a reduction in sample sizes.

There are a number of human trials planned or in progress of erythrocytic stage *P. falciparum* vaccines. Yesterday we heard about recent studies of SPf66. The group in Ifakara, Tanzania in collaboration with the Swiss Tropical Institute and the University of Barcelona have been conducting studies in Tanzania. The Institute of Immunology in Bogota is also conducting studies with SPf66 and other synthetic peptide vaccines. There is a study in progress in Papua New Guinea in which purified recombinant proteins based on three blood stage *P. falciparum* proteins are being studied in the field. There are also several Phase I studies of purified recombinant Pf MSP1 being planned in the United States.

I would like to tell you about a project called MuStDO 15.1, referring to the first iteration of a 15 gene approach to malaria vaccine development. MuStDO 15.1 includes DNA plasmids expressing the 5 genes that encode proteins expressed by irradiated sporozoites in hepatocytes, the plasmids from MuStDO 5.1. It also includes 10 genes encoding proteins expressed on the surface of merozoites or in the apical organelles. The hypothesis is that the pre-erythrocytic stage component will reduce the number of parasites emerging from the liver, and the blood stage component will prime the recipients' immune systems to the 10 erythrocytic stage antigens. Parasites emerging from the liver or from the first few cycles of the erythrocytic stage will boost these primed immune responses, and these boosted responses will limit replication of parasites from this infection, and thereby limit development of severe disease and death. These boosted responses will also limit replication of parasites from the next infection.

The only vaccine delivery system that we have available to us right now for doing this is DNA vaccines. Last year Sir Gus Nossal, chair of the Scientific Advisory Group of the Children's Vaccine Initiative wrote in Nature Medicine, "As arguably the most powerful development of all, DNA vaccines have made their explosive entry, possibly signaling a revolution in vaccinology based on their ease of production, stability and simplicity of combination." He didn't say anything about the immunogenicity of DNA vaccines, but rather stressed their simplicity which should allow for building the kind of complex vaccines that we think that we will need for malaria. In fact our work, and that of others indicate that DNA vaccines on their own are not the optimal way to induce any immune response. That doesn't mean that they won't be adequate; only clinical trials will provide the answer to that question. However, I believe that we must do better and while we are trying to improve the simple, naked DNA approach, we and others have moved toward a prime boost approach that is dramatically more immunogenic and protective than is DNA vaccination on its own.

Incorporating this complexity into a vaccine for humans requires a step by step approach starting with the simplest formulations and progressively making them more complex, if only for safety reasons. Our current work is in part based on some preliminary findings from a clinical trial that we conducted last year. In this study we showed that a DNA plasmid expressing the PfCSP was safe and well tolerated in volunteers (Wang *et al.* 1998). Furthermore, it elicited a $CD8^+$ T cell dependent, genetically restricted, antigen-specific

cytotoxic T lymphocyte response in 11 of 20 volunteers. This was not a malaria vaccine trial, but rather the first demonstration in normal, healthy humans that DNA vaccines were safe, well tolerated, and immunogenic.

We now have an international consortium working on the MuStDO 5 and 15 projects that includes scientists from the United States, Ghana, Australia, France, Panama, Peru and we hope, soon other areas. The cloning of the genes is taking place at the NMRC, Monash University in Australia, and in the case of PfLSA3 at the Institute Pasteur in Paris. Construction of the plasmids is at NMRC and Monash, and manufacturing at Vical Inc. in California. All of the genes used are based on the 3D7 sequence. However, the FVO sequence for PfMSP1 42 is also included.

The plan is to conduct phase 1 safety and immunogenicity trials almost simultaneously in the United States and in Ghana at the Noguchi Memorial Institute of Medical Research. If the vaccines are safe and well tolerated, we hope to conduct safety studies in progressively younger age groups at the Navrongo Health Research Center in Ghana, and do a phase 2a experimental challenge study in the United States, and phase 2b field challenge studies in children in Navrongo. We are hoping to begin the studies by the middle of 2000. We do not know when the studies will be complete, but if all goes extremely well, then we will have the results of the first field trials 4-5 years later. Many argue that there are still too many unanswered questions regarding the immunogens and the strategy, and we should delay initiation until we further refine both. However, we can predict learning a tremendous amount by starting now, but cannot predict what will we have in five years if we don't start now.

Before finishing, I would like to tell you about a third type of malaria vaccine development strategy. This approach is based on the idea of actually duplicating the immunity induced by exposure to the whole parasite (irradiated sporozoites or natural exposure). I have described to you an approach based on optimizing immune responses to 1, 2 or 3 of the proteins encoded by the estimated 6,000 genes in the *P. falciparum* genome, generally by a combination of recombinant protein or synthetic peptide and adjuvant. A second approach utilizes most of the known targets of protective immunity, and attempts to induce good immune responses against 15 of the proteins encoded by the 6,000 genes in the parasite genome, through DNA-based immunization. However, our human models are immunization with the whole organism either by natural exposure or by exposure to radiation attenuated sporozoites. It is possible that the strength of the immunity induced in these settings is dependent on immune responses to hundreds or thousands of parasite proteins. How do we get at this approach? There is currently a project to sequence the entire *P. falciparum* genome. The results of 3% of the genome have been published (Gardner *et al.* 1998), but we expect the complete sequence in 2-3 years. The question to grapple with now is how to adequately assess the thousands of new proteins to be identified in the genome project for potential inclusion in vaccines. We have proposed a strategy (Hoffman et al. 1998), and unfortunately I don't have time to go into it today. Nonetheless, I want to mention that I believe the integration of microbial and human genomics with molecular and cell biology, immunology and epidemiology in the next century will provide many of the answers to the questions we have been struggling with for so long.

In parallel, and perhaps more immediately, I think that several areas of field research could provide data that would substantially facilitate vaccine development. The most important is the identification of target groups for vaccines. As pointed out earlier these will differ from area to area. It is easier to immunize children than infants. Thus, if 2-4 year olds are suffering most from malaria, it does not make sense to immunize infants. To

achieve the most cost effective, efficient studies, it will be best to eliminate those who are not at risk. We need to have the smallest sample size as possible. We need to know if there are other groups, like those with sickle cell trait, who are at decreased risk who don't need to be immunized. We need to at least determine if there are outcome variables that can be measured that have a high predictive value for severe disease and malaria associated mortality. We need a more sophisticated assessment of the impact of bednets and other interventions on epidemiology, and the age-specific attributable reduction in mortality. Lastly, we need to develop better assays for predicting protective immunity. This will of course include a much more detailed characterization of the proteins and epitopes on these proteins involved in protective immunity. However, I believe that obtaining fundamental epidemiological data will have more of an impact on malaria vaccine development and design of field trials than will acquisition of immunological data or the mapping of epitopes.

The importance and difficulty of the task that lies ahead of us cannot be underestimated. Thirty seven years ago, malaria was a significant enough problem for the United States along with many other countries to release stamps commemorating attempts to eradicate malaria. That was more or less the same time that President Kennedy vowed to put a man on the moon. Thirty years ago the first man walked on the moon, but we are still a long, long way from eradicating this deadly disease. I believe that development of malaria vaccines will be critical to actually realizing the dream of eradicating malaria.

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Correlates of Immune Protection: Practical Implications

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The ultimate test of a vaccine against *P. falciparum* malaria is its actual effect on human beings. From this basic consideration, we inferred that long term immuno-epidemiological studies are the most appropriate means of understanding the critical characteristics of the interactions between parasite and man, and a prerequisite to the development of a malaria vaccine.

Only extremely well documented situations of clinical resistance or susceptibility to malaria, and associated immune responses to *P. falciparum* parasites in endemic areas, can be expected to give accurate and reliable data. This means, in particular, that only active, daily and carefully planned, controlled and long-lasting investigations in selected endemic areas can be of value. Such investigations are critical if one expects to characterize the essential immune responses involved in the development of protection.

We initially decided to spend time, energy and money in such a study including the active and full time participation of specialists from different origins. The common goal was to understand, analyze and literally dissect every single malaria-related event occurring in Dielmo, a small village of Senegal where malaria is holoendemic. A staff of medical doctors, nurses, and scientists (including epidemiologists, entomologists, and immunologists) was established. This long-term study involved 250 villagers, who were included after informed consent, which was annually renewed. This program was designed so as to accurately identify any single episode of fever and disease whatever their origin, in every family, everyday all year round. In practical terms, this means that highly trained medical staff were permanently stationed in the village itself, ready to handle any complaint of a villager at any time, during day or night. In addition, active detection of symptoms was recorded by daily visits to every single household. Only such a daily visit to each inhabitant can provide a reliable indication of the actual occurrences of sickness in the village. Every month, a capillary sample was obtained from each inhabitant of the village. In parallel, entomological data are recorded every month, all year round.

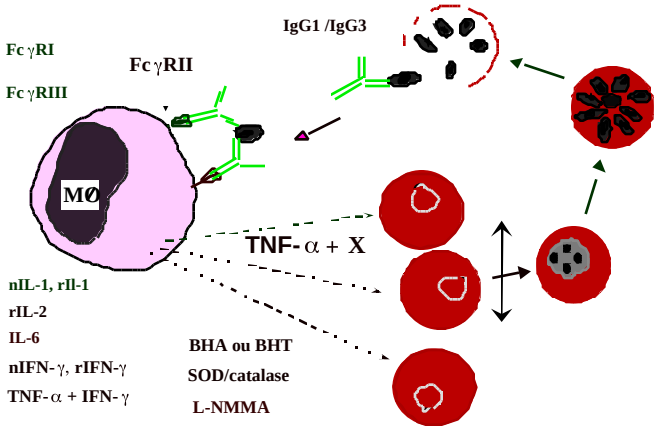
Analysis of the daily data from the first 3/4 years of study allowed us to establish an age-dependent threshold level of parasitaemia associated with clinical malaria. It is our firm conviction, after almost ten years of follow up in this village, that only the conjunction of the permanent presence of a medical staff, the active enrolment of the participating villagers for the daily search for sick persons, and above all, the constant approval of the villagers, guarantee the validity of such data gathering. Hence, we also believe that despite its cost, the acute value and unique quality of epidemiological indications, regularly checked and controlled in this program, offers a trustful basis for determining the clinical status of an individual and allows us to compare biological tests between inhabitants of the village.

The experimental transfer of IgG antibodies from protected Africans to unprotected children has allowed us to better understand how these antibodies act upon the parasite. Of particular interest, is the observation that antibodies reduce parasitaemia, but do not eliminate the parasite (i.e. there is non-sterilising immunity). When tested *in vitro*, immunoglobulins were not inhibitory on their own: antibody-dependent inhibition of *in vitro P. falciparum* cultures was only observed when monocytes were present. Thus,

protective antibodies are apparently acting indirectly by triggering the release of parastatic substances from monocytes. This mechanism, known as antibody-dependent cellular inhibition (ADCI) (Figure 1), operates via cytophilic antibodies only (i.e. IgG1 and IgG3). These original observations formed the basis of detailed field research to evaluate the respective roles of antibody subclasses, in response to parasite antigens in different endemic areas of Senegal.

1) In Dielmo, we found that antibodies against whole blood stage parasites increased with age, and hence with cumulative exposure to *Plasmodium* and decreasing risk of malaria attacks. In a cross-sectional study, the association of antibody responses against parasite antigens and occurrence of malaria attacks was analyzed (Figure 2). This study showed that, of the different antibody classes and subclasses tested, only the IgG3 antibody reactivity was significantly associated with a decrease in the risk of malaria episodes when all known confounding variables were controlled(age, G6PD deficit, AA and AS Hb phenotype). The role of the IgG3 antibody reactivity was more pronounced in young children than in adolescents, and comparatively reduced, but still present, in adult individuals.

Figure 1. The ADCI mechanism



2) On the basis of these initial observations, we then tested the antibody activity detectable in different groups of villagers, differing by their relative susceptibility to malaria attacks. For example, among women, the risk of malaria attacks was increased 4-5 fold during pregnancy. At the same time, we found a drop in IgG3 antibody reactivity against the whole parasite. In a subgroup of children, we found individuals repeatedly suffering from malaria attacks, whereas another subgroup of children of comparable age had no such risk of malaria. In these two situations, parasite-specific IgG3 antibody activity was consistently decreased when the risk of malaria attacks was raised. Therefore, in different situations of susceptibility/resistance to disease, a key role for specific IgG3 antibody reactivity was observed.

3) During the period following delivery (post partum period), a drop in IgG3 was observed for a period of up to 3 months in all women tested. The risk of malaria at this time was significantly increased (around 7 times) by comparison with the risk found in the same women tested one year later, in similar conditions of parasite transmission levels. This was another situation where alteration in IgG3 levels was associated with an increased risk of malaria.

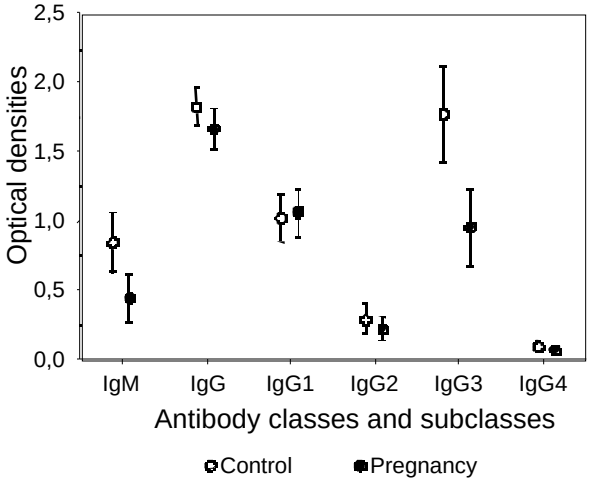


Figure 2. Pattern of parasite-specific antibody activity during pregnancy and comparable control periods in Dielmo

4) The antibody response against different malarial antigens (MSP1, MSP2, MSP3, AMA1, RESA) was then tested in Dielmo. Only the antibody responses against the recently described Merozoite Surface Antigen 3 (MSP3) (Figure 3) were related to protection. In Dielmo, IgG1 and IgG3 responses to MSP-3 increased with age, and hence with cumulative exposure to *P. Falciparum* (see below). The ratio of cytophilic to non-cytophilic antibodies was also evaluated: for each age group (i.e. in an age-independent manner), this ratio was higher for individuals with no malaria attacks than for individuals who had suffered from malaria attacks (Figure 4).

5) IgG3 antibody responses against different antigens (MSP1, MSP2, MSP3, R23, GLURP, SERP) were then tested in cord blood. Different levels of antibodies were detected, and the occurrence of malaria attacks varied between infants. For some children (n= 18), the first detectable infection by *P. falciparum* led immediately to a malaria attack. In contrast, for other children (n= 21), a peripheral parasitaemia was detectable for a mean of 27 days before the occurrence of their first malaria attack. These two groups of infants belonged to mothers with significantly different levels of anti-MSP3 IgG3 antibodies. In this natural situation of parasite-specific antibody transfer, there was a direct association between the transfer of maternal anti-MSP3 IgG3 antibodies and a delay before occurrence of a malaria attack in infants. The *Plasmodium*-specific antibody responses measured in the cord blood were therefore related to resistance to malaria during the first 3 months of life.

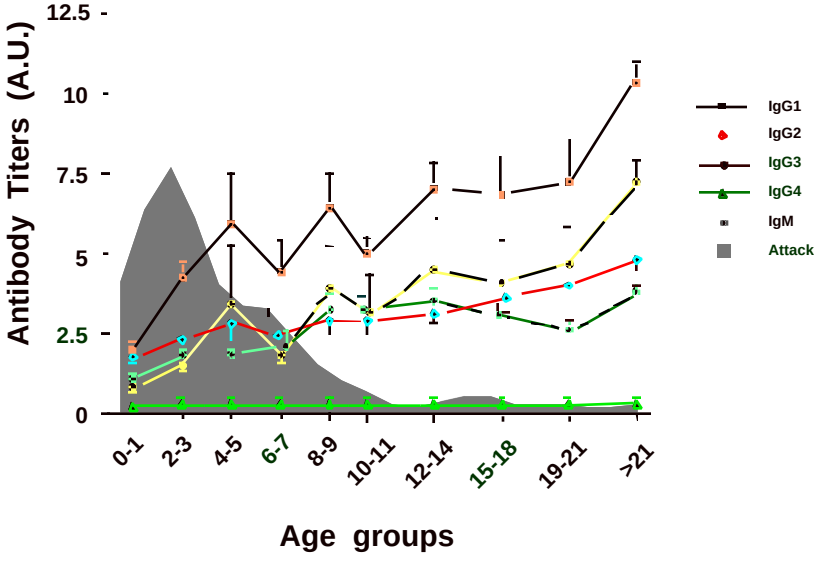


Figure 3. MSP3-specific antibody responses in Dielmo (holoendemic area)

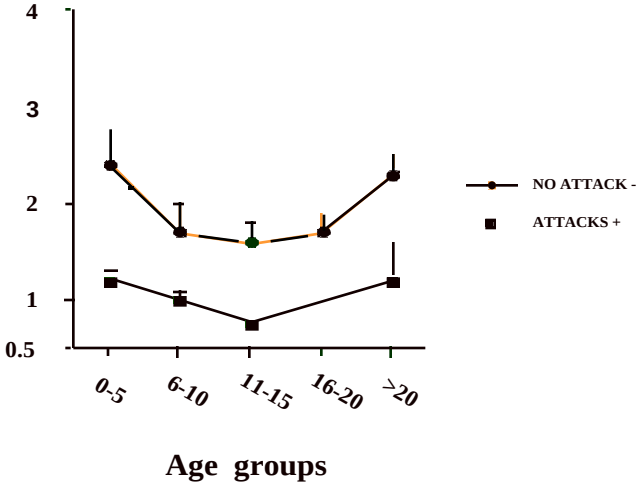
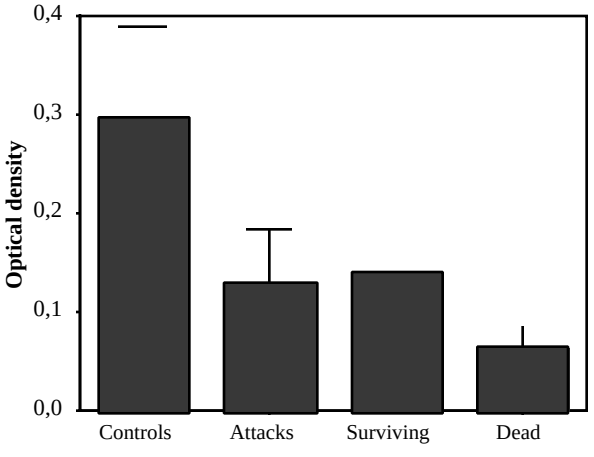


Figure 4. Ratio of cytophilic to non cytophilic anti-MSP3 responses in Dielmo

6) When the antibody reactivities of patients from Greater Dakar with cerebral malaria were evaluated, it was found that the level of IgG3-specific activity was significantly associated with an increased chance of recovery from this life-threatening episode**.** When the antibody response against MSP3 was evaluated, we found three times more IgG3 anti-MSP3 in the subgroup of patients with a propitious evolution as compared to the subgroup with a fatal evolution.

7) Finally, a prospective study was carried out during the low transmission period in a seasonal malaria transmission area (the village of Niakhar) (Figure 5). Blood was obtained on capillary samples from 4,200 children at the time of no malaria transmission. The children were followed up during the following transmission season, when 51 suffered from severe malaria. The level of IgG3 activity directed to MSP3 was found associated with both a decreased risk of severe malaria attack during the following high transmission period, and an improved prognosis following drug treatment in 42 out of 51 severe malaria episodes.

Figure 5. Pattern of antibody activity against the MSP3 antigen in Niakhar (mesoendemic area)



These observations were therefore convergent. They consistently illustrated the association of IgG3 antibody response to antigens of *P. falciparum* and in particular MSP3 with a reduced risk of malaria.

Thus, both immuno-epidemiological investigations and IgG transfer experiments highlights the unique role of cytophilic antibodies in the control of human malaria. This convergence cannot be fortuitous, but most likely reflects one of the mechanisms of defence developed by human beings naturally exposed to *P. falciparum,* and actually involving the participation of cytophilic antibodies.

The mechanism of Antibody Dependent Cell Inhibition (ADCI) is one of the prime potential processes involved in protection against malaria, and more particularly in premunition. The merozoite surface protein, MSP3, was identified by Claude Oeuvray using ADCI as a functional screening tool. Of particular interest, was the fact that MSP3 was also the most readily recognized of the antigens tested in different situations in Senegal. IgG3 activities directed against the MSP3 antigen were found to be critically involved in individuals with a marked protective status. It was not unexpected that an antigen characterized on the basis of its capacity to be a prime target of ADCI (a mechanism associated with an isotype imbalance) was predominantly recognized by cytophilic

antibodies in protected versus non-protected individuals. This was basically an *in vivo* confirmation of *in vitro* data.

The IgG3 activity has been consistently and repeatedly found associated with a markedly reduced risk of malaria attack. This suggests that it could be considered as a prognostic indicator of resistance to malaria attacks in endemic areas. It is a privileged biological marker of protection in different situations of resistance or susceptibility to malaria attack.

In summary, of all the antibodies tested against several erythrocytic stage antigens, only those with a specificity for MSP3 were found significantly associated with a reduced risk of malaria attack. The information initially obtained from the IgG transfer experiment has emphasised a critical role for the co-operation between antibodies and monocytes and led to the identification of the MSP3 antigen. We now confirm in different epidemiological and clinical situations a critical role for the cytophilic antibody subclasses, particularly those directed against MSP3. It is clear that studies on human beings, the natural host of *falciparum* malaria are of utmost value and, despite their huge implications in term of cost and constraints, such approaches probably represent the most rewarding and informative way to gain insight into host-parasite relationships and ultimately efficiently fight this deadly disease. It came as a satisfactory and encouraging result that data obtained from two different lines of research were so strongly convergent and highly complementary. The above studies provide well-established markers of protection in humans and we believe they will be of paramount value in vaccine development.

What can we learn from Molecular Epidemiology?

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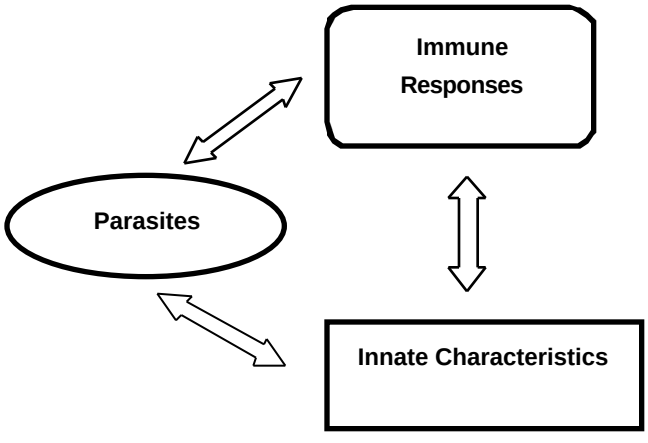
During this talk, I will try to give a brief overview of what we have learnt from molecular epidemiology that is relevant to vaccines, as this was the task that our Chairman assigned to me. The outcome of an infection depends on a complex matrix of interrelated factors (Figure 1):

- The host's innate characteristics, such as genetic susceptibility, age and nutritional status.
- The host's immune response, including the immune status at the time of the infection, and the magnitude and type of response to the infection. This response is crucial in the determining whether immunopathology or protection results and it is dependent upon factors such as previous exposure to *P. falciparum* parasites, on past or current infections and on the age of the host.
- The parasite itself, including its phenotypic characteristics, antigenic makeup and multiplication rate. The actual number of parasites present and the number of distinct genotypes are also important.

Figure 1. Matrix of factors



The respective weight of each parameter is itself strongly influenced by transmission intensity and duration. It makes a difference if you receive all your bites in a two month period or if you get them all over the year. What we are trying to do in molecular epidemiology is to integrate the dimension of parasite characteristics and diversity into the equation. What I shall do now is concentrate on the parasite/immune response, as this is the most crucial aspect for vaccines.

Parasite Diversity

Three major factors contribute to parasite diversity:

1 Allelic polymorphism. Many genes coding for surface antigens, such as merozoïte or sporozoïte surface antigens, show extensive allelic polymorphism. This results in numerous serotypes and T-cell epitope variants within the population.

2 Antigenic variation. The parasite genome has a repertoire of 50 *var* genes, each coding for different serotypes of an antigen exposed on the surface of the infected red blood cell. This results in a phenotypically heterogeneous population of otherwise identical parasites, such that no single infection is homogeneous for its red blood cell surface phenotype. Not only are there 50 *var* genes per genome, but the *var* repertoires within the species are highly diverse, creating a very large population diversity.

3 The sexual cycle. Sexual reproduction in the mosquito can potentially generate novel chromosome assortments, gene combinations and alleles from heterozygous oocysts. The very existence of sexual reproduction in a highly polymorphic species is worrying in the long-term, because it is able to generate an endless source of novelty.

These factors all contribute to diversity of field populations so that parasite isolates can differ in:

- surface antigen serotype
- combinatorial association of surface antigen
- variant antigens expressed at the time of sampling
- their *var* repertoires
- the number of clones present in each isolate.

Molecular epidemiology started long ago, in the 1970's, with the work of Carter, McGregor and Voller. Using isoenzyme typing they discovered several important features concerning *P. falciparum* infections in man, which have been largely confirmed by subsequent molecular epidemiology studies using either monoclonal antibody typing or the more widely used PCR approach.

PCR genotyping

The PCR genotyping strategy has become popular, because it presents several advantages:

- It is very sensitive, much more sensitive than the microscope, allowing analysis of asymptomatic infections.
- It is not restricted by expression stage - with one parasite DNA sample collected from peripheral blood, mosquito or an autopsy specimen you can analyse virtually all genes of the parasite, whatever their stage of expression.
- It generates the material to be studied, instead of using it up as with other techniques, providing the opportunity to study novel alleles by DNA sequencing. So the more PCR you make and sequence, the more you know about diversity.

However, PCR genotyping has its limitations and constraints and is far from a perfect tool. For example, quantification is problematic in isolates with multiple clones, and minor alleles are frequently undetected. It is also at present impossible to discriminate gametocytes from asexual parasites, as they have the same genotype. This is a real issue when one studies the dynamics of infections in man. In addition, PCR analysis concentrates on genotypes without providing any clue on the phenotypic consequences: two alleles with different repeat copy numbers will be typed as genetically different, but will probably express the same serotype. Finally, unlike mAbs, negative parasites are not visualised. If an allele has a mutation in the sequence of the primers, then it will not be amplified and remain undetected.

Cross-sectional studies

Cross-sectional studies allow study of genotypic diversity within parasite populations and individuals, as well as some characteristics of infections such as 'complexity' (the number of distinct genotypes present in an isolate).

We have tried to understand whether there are geographical and temporal variations and what the parameters influence allelic distribution and complexity, in particular age, innate susceptibility and immune responses.

Let me now describe the main findings. I hope the audience will forgive me if I take examples from our own work. I use these because of convenience, but let me say that we all find the same things provided we compare what is comparable. There are a few discrepancies in the technical details used, but overall the findings in one holoendemic area are indeed observed in another one, and findings in different mesoendemic areas are extremely consistent.

Population diversity

Most data I will show today concerns a PCR analysis of field isolates using the most popular genetic markers for assessing polymorphism, namely the gene coding for the merozoïte surface proteins, msp 1 and msp2. It shows a very large allelic polymorphism, allowing easy discrimination of isolates based on the polymorphism of these 2 loci. These polymorphisms "flag" each isolate and provide a sort of surrogate estimate of the extent of parasite diversity within the population.

We have done an analysis of parasite diversity in Dielmo and Ndiop, two Senegalese villages located 5 km apart, and in Dakar (Figure 2). The latter has urban malaria in a hypoendemic region, with people being infected elsewhere in Senegal when they visit their parents. We have also done studies in Madagascar with Ronan Jambou, and in French Guyana with Frédéric Ariey on the other side of the Atlantic Ocean.

Figure 2. The villages of Dielmo and Ndiop in Senegal

Allele numeration

It is clear from the results in Table 1 that there are a very large number of alleles in Dielmo, Ndiop and in the isolates collected in Dakar. In contrast, in French Guyana where there is hypoendemic malaria with low transmission and low incidence, parasite diversity is much, much lower. French Guyana is a very interesting place in terms of population genetics.

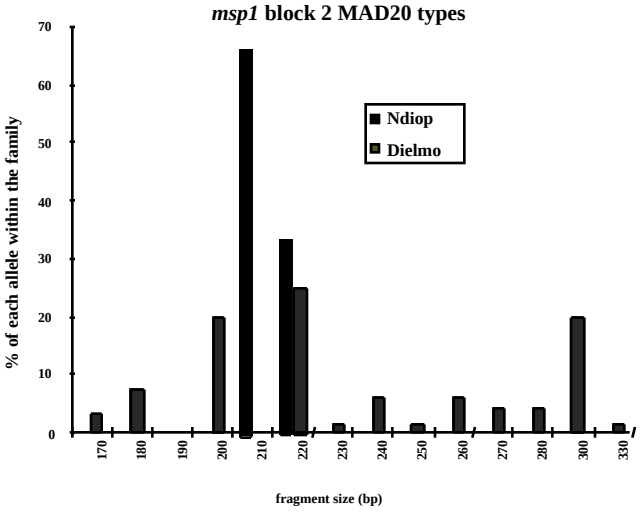
Table 1. Population diversity: number of *msp1* & *msp2* alleles detected in cross-sectional surveys

	Ndiop	Dielmo	Dakar	French Guyana
Number of isolates	125	144	86	125
Msp 1 bl2	13	33	19	4
Msp 2	27	47	31	2

Table 1 shows the number of distinct *msp 1* and *msp 2* alleles, identified by size polymorphism within each allelic family. This is a minimal estimate, because alleles with identical size may have point mutations that remain undetected by this approach.

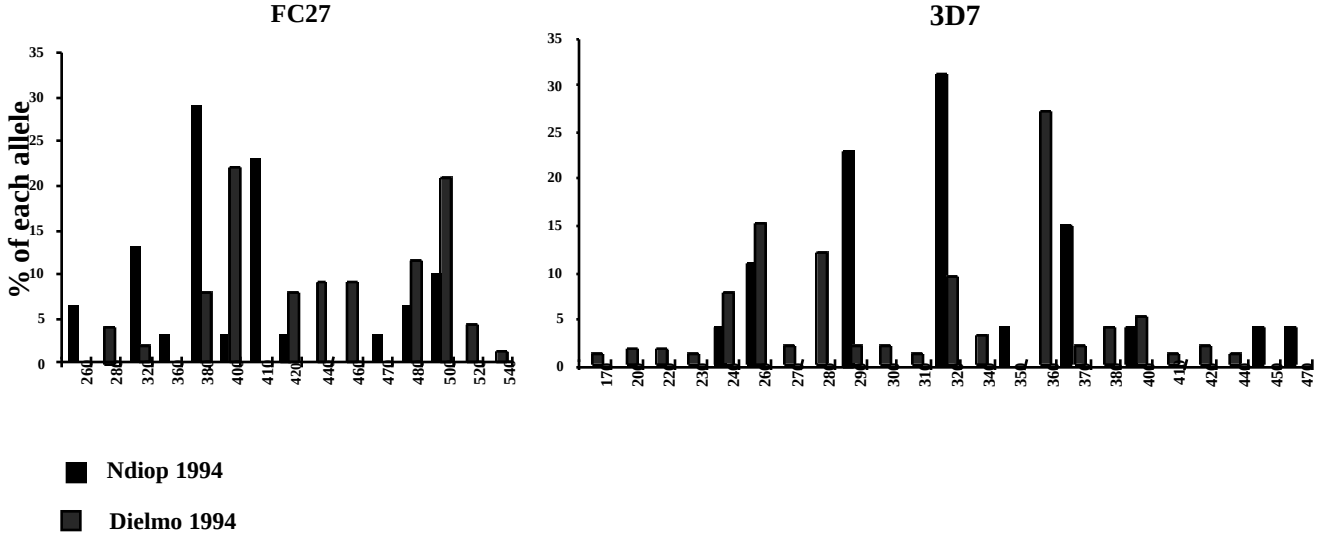
So the first message is that parasite diversity is large, but not everywhere, depending upon the endemicity. There is no linear relationship with the transmission level, but clearly the most diverse population is Dielmo, where transmission is highest.

Figure 3. Distinct individual *msp1* allele distribution in Dielmo and Ndiop, October 1994



Is there geographical variation ?

For us the answer is yes. We have compared the alleles present in Dielmo and Ndiop (approximately 40% of the inhabitants were sampled) in a cross sectional survey conducted in parallel in both villages in October 1994 (Figure 3). This clearly shows major differences in allele distribution. Here is the distribution in both villages of individual *msp 1* block 2 alleles of the Mad20 family. What you see is that the dominant alleles in Ndiop are absent from Dielmo and vice-versa. The same is true for the individual msp2 alleles of both sub-families, the major alleles in one place are minor or virtually undetected 5 km apart (Figures 4 & 5).

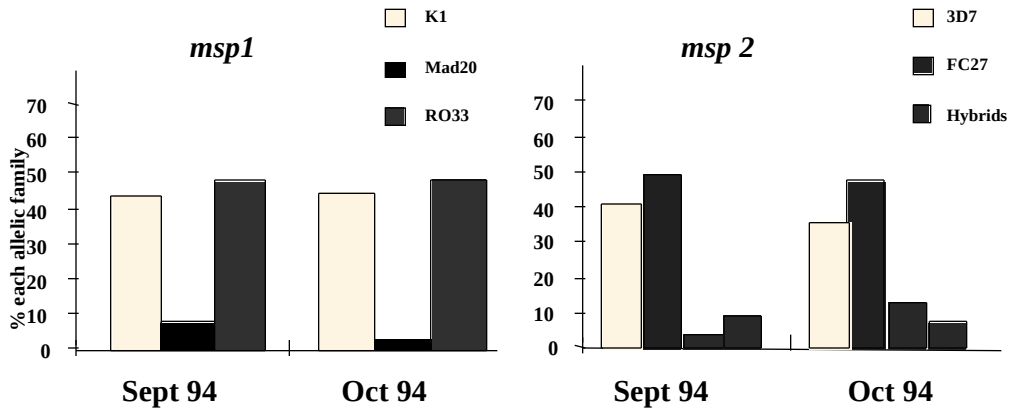


Figures 4 & 5. Distinct individual *msp2* allele distribution in Dielmo & Ndiop, October 1994

Is there variation with time in one place?

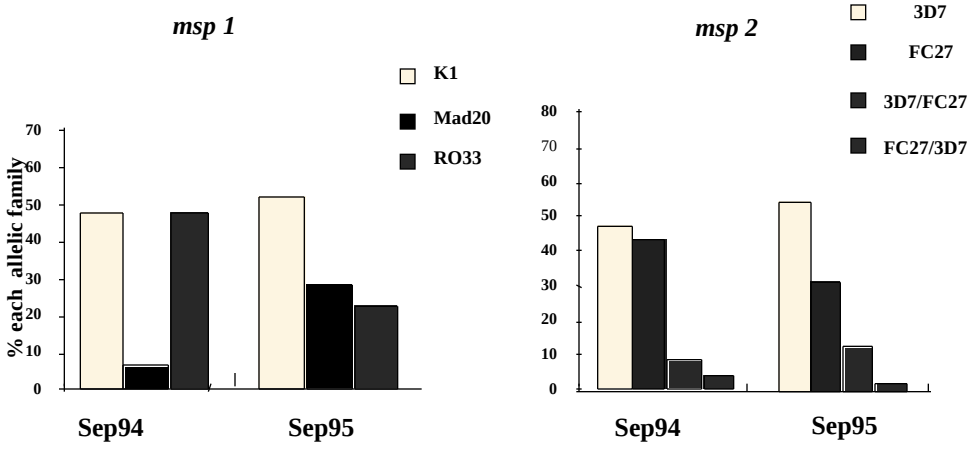
We conducted a series of cross-sectional surveys in Ndiop over a one year period. We first compared two surveys conducted 1 month apart during the 1994 rainy season, namely at a time where parasites are actively transmitted from one person to the next. This showed that allelic distribution in September and October was similar (Figure 6 & 7), reflecting active circulation of genotypes within the village (if one genotype identified in a person in September is no longer in that person in October, it will be found in another person).

Figures 6 & 7 Ndiop: Stable *msp1* & *msp2* allelic family distribution during the rainy season



We also conducted a series of surveys during the following dry season and up to the next transmission season in September 1995. This showed that the parasite population of 1995 was totally different from the parasite population circulating in 1994 (Figures 8 & 9). This was due to substantial variations occurring during persistent chronic carriage in the dry season. I'll describe later what happens during the dry season. So there is substantial year to year variation, at least in this place.

Figures 8 & 9. Ndiop: year to year variation of msp 1 and msp 2 allelic family distribution



In summary, the message is that field parasite populations are very polymorphic and there is extensive allelic polymorphism. Our own analysis of *var* repertoires has so far shown that they are very diverse and this is what the professionals of *var* say too. We have found, and others have also, substantial geographical micro-heterogeneity, as well as temporal variation in allele frequency.

What are the consequences of these findings for vaccination ?

We need to consider local diversity of vaccine candidates in relation to two particular issues:

1. We need to know **more** about diversity, which in practice means sequencing of a very large number of alleles from representative samples of the local population. The big difficulty here is what the word “representative” means and what the word "local" means: does it mean Dielmo or Ndiop? A specific geographical zone? Senegal? West Africa? We need to know much more about what a “population” is for these parasites.
2. The second thing is that we need to understand the **consequences** of such diversity for the immune system. This is a large undertaking and the picture indeed may be quite different for vaccine-induced immunity and for naturally acquired immunity. But we have to study both.

I have unfortunately no time to talk about this. Adrian Hill’s group in Oxford has investigated this aspect for T-cell epitopes, in particular CTL epitopes of the Circumsporozoite protein in man. The outcome is that the epitope diversity is a source of big trouble to existing and future responses.

We have addressed the issue for blood stages, both for antigenic variation and allelic polymorphism, using experimental infections of *Saimiri* monkeys with identified antigenic variants of one parasite line or with different parasite strains. The message is that we have to worry, but not panic. Variant-specific immunity is relayed by recognition of conserved antigens. What seems to be another piece of cake is when we start inoculating several strains and make multiple infections (which is extremely frequent in endemic areas as we will see shortly).

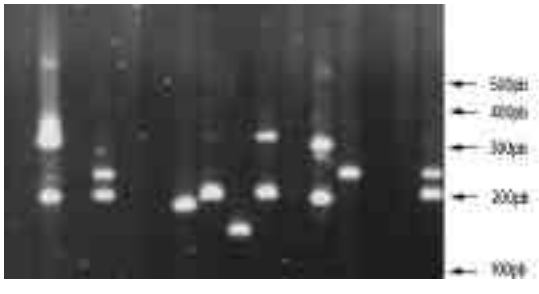
We have also addressed this in the villagers of Dielmo and Ndiop. Hélène Jouin has investigated the allele-specific response against *msp1* block 2 (see poster abstract). Here too the message is that immune responses is specific, but this is not too worrisome as many variant linear epitopes are shared by many alleles, which are built up as mosaics of variable epitopes just like a Lego game with little bricks.

Challenge by heterologous parasites is likely to happen in most endemic areas. In my opinion, protection against heterologous parasite types should be one of the stringent criteria to be used early on in vaccination trials and in preclinical studies.

The last point is obviously that when parasites do come up in vaccines, they should be typed. The gene or the genes coding for the antigen(s) included in the vaccine should be sequenced to look for possible escape mutants.

The second aspect that I will briefly summarise for you relates to infection complexity. Figure 10 illustrates a typical agarose gel where a series of samples has been analysed for *msp1* block 2 polymorphism. As you can see, some samples generate more than one band. As there is only one copy of the gene per genome, the detection of more than one band is synonymous with more than one genotype in the isolate.

Figure 10. Agarose gel analysis of size polymorphism



Many isolates contain more than one genotype The proportion of isolates with multiple genotypes varies in different endemic areas. For instance, in Dielmo where malaria is holoendemic, almost 100 % of the asymptomatic infections contain multiple bands, but only 50% of the isolates in Ndiop where malaria is mesoendemic (Figure 11).

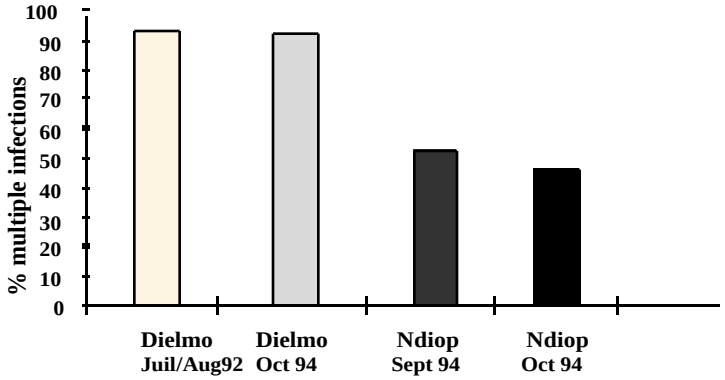


Figure 11. % Multiple infections in asymptomatic Ndiop and Dielmo villagers (transmission season)

Not only is the proportion of isolates with multiple infections different, but also the average number of genotypes per isolate differs. In Dielmo this is about 3.5, whereas in Ndiop this is 1.5. This is true for both surveys conducted in both villages (Figure 12).

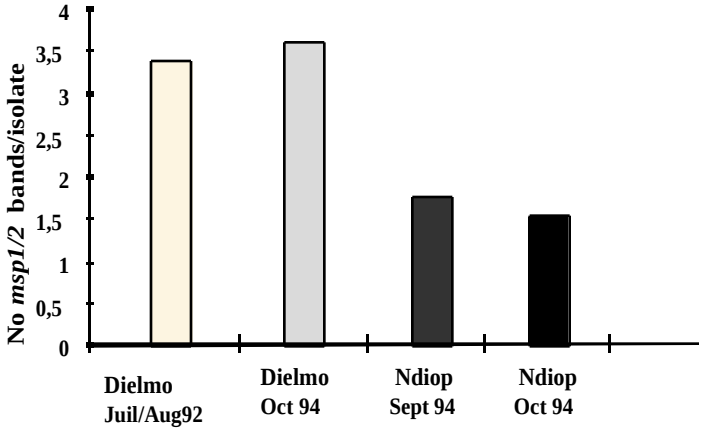


Figure 12. Infection complexity in asymptomatic villagers in Ndiop and Dielmo (transmission season)

Factors influencing complexity

I have no time to go into details on the factors influencing complexity and a special issue of the Transactions has just been published under the auspices of the Swiss Tropical Institute. However, to summarise the key points: complexity is influenced by transmission intensity, parasite density, age in holoendemic but not in mesoendemic areas, and by treatment.

Longitudinal studies

Let me now move to what we have learnt from longitudinal molecular epidemiology studies and the implications of the results for vaccines. Longitudinal studies have provided new insights into the dynamics of infections, on the factors which contribute to the occurrence of clinical attacks and on what it happening during chronic and asymptomatic infections.

In Dielmo, Jean-François Trape and Christophe Rogier have conducted an extremely well-documented longitudinal survey during the 4 months of intense transmission of the 1990 rainy season, with daily monitoring of clinical symptoms and measurement of parasite density 2-3 times a week.

Here is an example of a child who has suffered three clinical successive attacks of malaria within a period of 3 months (Figure 13). The parasite density is recorded above and the body temperature below. Each rapid steep increase of parasite density is associated with clinical symptoms, which are then treated.

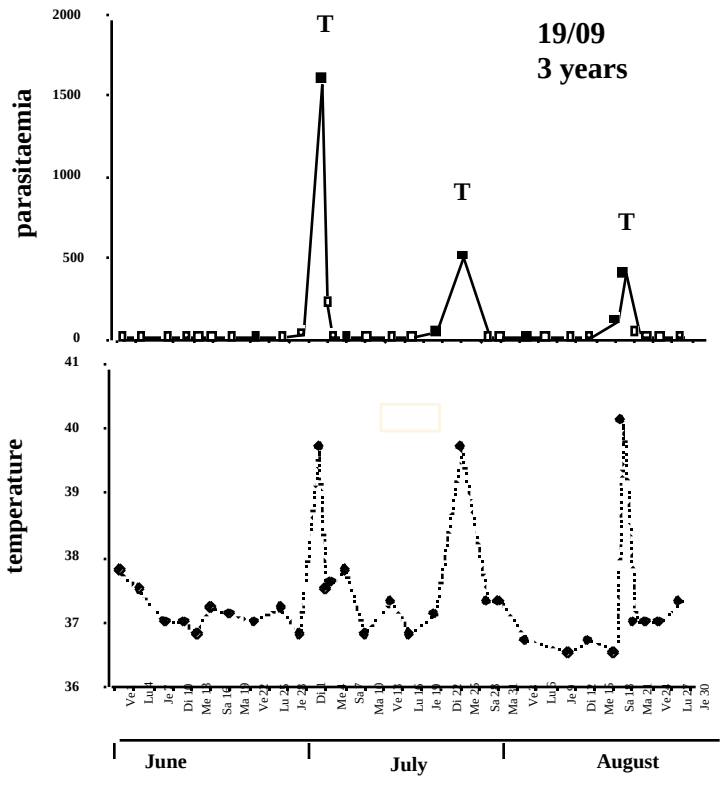


Figure 13. Childs successive clinical attacks within three months.

We genotyped parasites collected from the children during each clinical episode and systematically every second week. What came out was a very clear cut conclusion. The parasites collected were different for each episode. The DNA collected systematically and that collected four days later during the episode were genetically identical, indicating that those parasites that were present at low density on one day multiplied to high density and provoked the episode.

The conclusion that parasites collected during successive clinical episodes experienced by children are genetically different was valid for all cases, except when there was a recrudescence due to incomplete treatment. Such clear cut infections are not always the case. There were other cases where the dynamics of infections were more complex, with alternating symptomatic and asymptomatic phases. However, the conclusion that I just stated remains true: for each clinical episode which required treatment, parasites were genetically different, and clinical episodes were caused by parasites that multiply very quickly.

However, not all -new infections end up in a clinical episode. You see here that the parasites collected from this child do not reach a very high density and are controlled. The parasites differ from those collected 6 days later during the second episode.

To summarise, what we have learnt is that clinical episodes are associated with rapid multiplication of recently inoculated parasites which reach high density (above a pyrogenic threshold, which is dependent upon endemicity). Clearly the parasites collected during successive clinical episodes experienced by these children over this four month period were genetically different (Figure 14). The other noticeable observation is that children control multiplication of some genotypes (and then the infection is asymptomatic), but not of others which grow fast, passed the threshold density and cause a clinical episode.

In terms of vaccination, this indicates that **control of the parasite multiplication rate** is an essential component of protection against clinical malaria and sterile immunity may not be a pre-requisite. It looks as if to reduce the multiplication rate is enough to slow down the pace of infections and provide time for the immune response to become effective. All trials that have been done in animal models have looked for a 'yes' or 'no' reply, and have aimed at inducing a golden, sterile immunity. Reducing growth rates from steep curves to flatter ones may be sufficient to prevent symptoms and to leave the time for protective responses to take over and do their job.

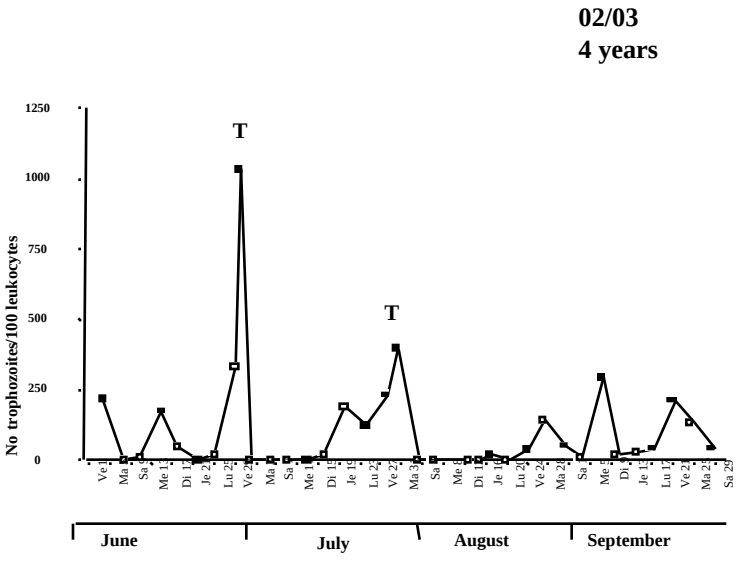


Figure 14. Clinical episodes over four months

In the last part, I want to briefly address the issue of infection dynamics in asymptomatic subjects. I think there is confusion here and we need to distinguish between occurrences in different transmission conditions. The situation differs in people who are frequently superinfected, during heavy transmission periods in holoendemic areas, and in those with chronic infections in the dry season, when there are no mosquitos around, and in mesoendemic areas, where people receive ten or fifty times less infections than in holoendemic areas. Findings in holoendemic areas cannot be extrapolated to other places.

In brief, the conclusions for infection dynamics in asymptomatic subjects are as follows. When parasites are actively transmitted and novel inoculations occur at high frequency, we observe a rapid turn-over in the peripheral circulation. This has been so far observed only in holoendemic areas.

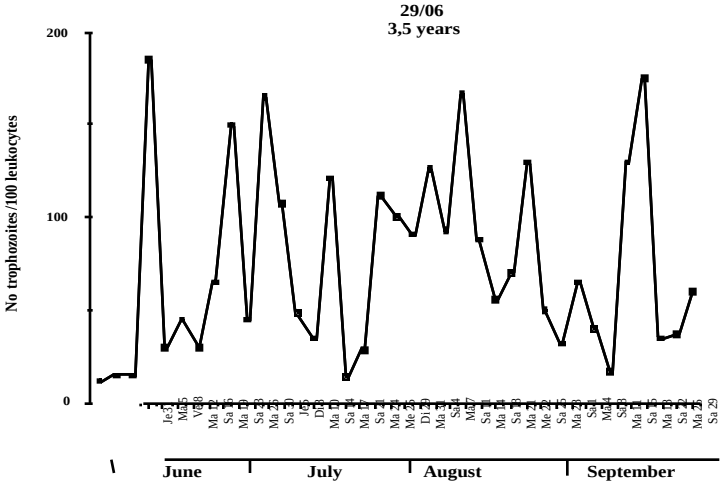


Figure 15. Parasite record of untreated girl from Dielmo

When there is no transmission, during the dry season, then the situation is very different. Prolonged carriage of single clone infections occurs, or alternatively, if the dry season starts with a multiple clone infection, then we observe fluctuations in the various parasite types.

Figure 15 is the parasite records of a 3,5 year old girl from Dielmo collected over 2 months during the same 1990 rainy season. She remained untreated throughout this period.

We analysed five isolates collected from this child. Each one generated a distinct pattern, indicating a rapid turnover of parasites in the periphery (Figure 16). These samplings were done approximately every second week (day 1, day 16).

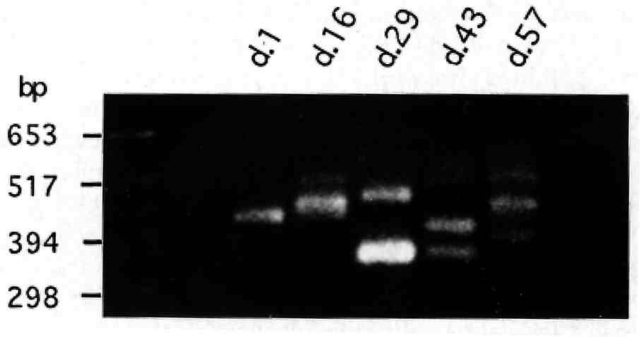


Figure 16. Base pairs at two weekly intervals

We then studied more precisely the parasite dynamics and collected blood on even or uneven days, in order to investigate parasites that were sequestered the day before. Turnover of parasites was again observed, with parasites detected on days 1 - 5 being "replaced" by another population (Figure 17).

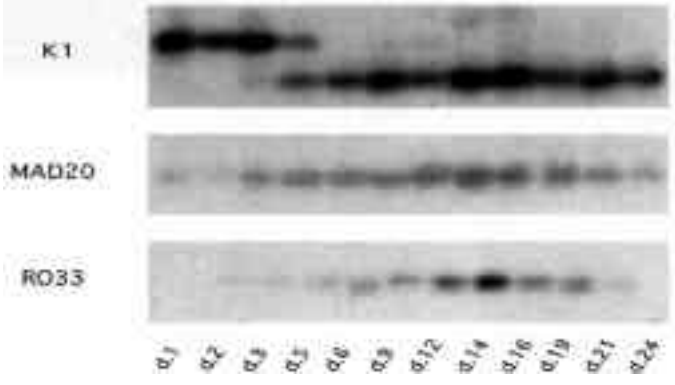


Figure 17. Parasite dynamics over odd and even days.

Similar data have been observed in a holoendemic village of Tanzania by Anna Farnert, Georges Snounou and Anders Bjorkman. There are daily fluctuations of the genotypes circulating in the peripheral blood.

No doubt when transmission is intense, infections are complex and there is a rapid turnover of the population in the periphery. Dominant parasites change frequently. We have calculated that a specific genotype was observed for about 2 - 3 weeks.

The opposite is observed in places where transmission is interrupted and people carry single clone infections. Pierre Daubersies has studied chronic infections in Pikine, a locality close to Dakar where malaria is hypoendemic. The same clone was observed throughout the survey (5 weeks), indicating stable carriage.

As I mentioned before, we have recently conducted an analysis of chronic carriage at the village level during the dry season in Ndiop. Figure 18 is data from Didier Fontenille and his colleagues showing the entomological inoculation rate, which has been monitored monthly throughout this three year period.

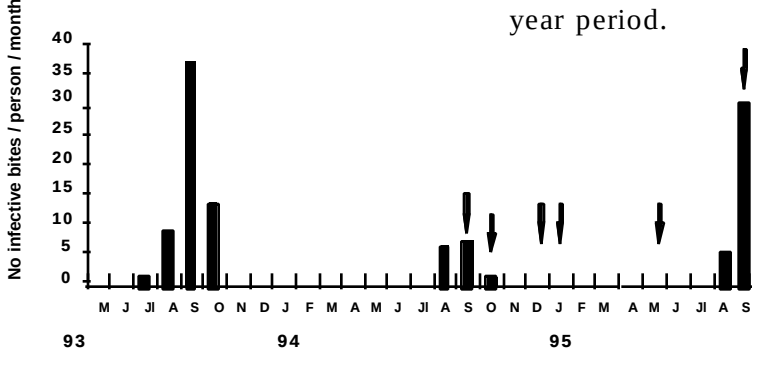


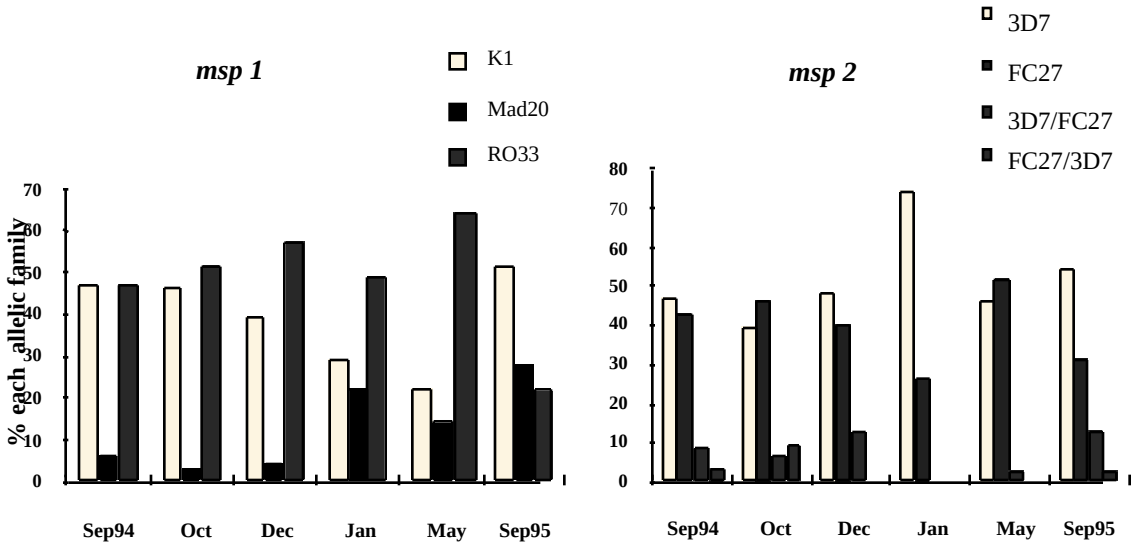
Figure 18. Entomological inoculation rate in Ndiop

We have observed 2 things. Firstly, there was a

considerable temporal variation of the parasite population during the dry season when no mosquitos were captured. This resulted in the September 95 population being very different from the September 94 one, as I mentioned earlier (Figure 19). Only one out of 46 individuals studied had the same single band genotype throughout.

All the others had a genotypic profiles that differed from those detected in the earlier surveys. This is not due to novel inoculations. We think that this reflects a major variation of the dominant population infecting a person upon prolonged, chronic carriage: parasites that were barely or not detected at the onset of transmission take over progressively to become the dominant population after a few months.
Similar fluctuations have been observed previously during the dry season in Sudan in two different villages. It therefore looks as if there are major changes in the population as a whole during chronic carriage where serious selective forces are obviously opposing the parasite.

Figure 19. Ndiop: temporal variation in msp1 and msp2 allelic family distribution



The second very interesting and unexpected finding was that after seven months of undetected transmission, parasite prevalence significantly drops in younger children. Figure 20 shows the prevalence in children under 7 years, in 7-14 year-olds and in those above 15 years. The prevalence drops progressively upon prolonged absence of inoculation in the youngest children. This is quite amazing as children are thought to have the least efficient anti-parasite immunity. These of course are untreated villagers.

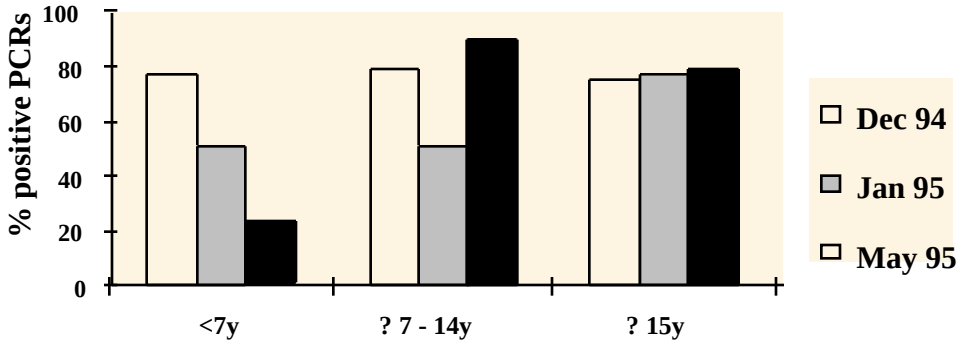


Figure 20*. P. falciparum* prevalence by age during the dry season in Ndiop

So interesting things happen during the dry season:

- There is a specific reduction of prevalence in younger children. Whether this reflects total elimination or simply a substantial reduction in density in younger children is unclear.
- There are also major changes in allele distribution during the dry season.

This ends up with an interesting picture which makes sense: young children at the onset of the next transmission are predicted to have a reduced concomitant immunity. They have an increased susceptibility to infections due to decreased clinical immunity and to the fact that their anti-parasite immunity is limited. If on top of that the face of the parasites has changed during the dry season, then this further increases their risk of a clinical episode once they get infected during the next transmission season.

Conclusions

In conclusion we have learnt that :

- Field parasite diversity is large in most places.
- Heterologous challenge is likely to be the rule. This means that the immune system is frequently faced with novel antigen combinations, and even conserved epitopes are exposed to the immune system in novel contexts, because they are associated with variable determinants.
- Mixed infections with multiple clones are very frequent, including in places where transmission is moderate or low, and this is somewhat puzzling.
- Parasite densities and type fluctuate, which means that allele ratios vary with time, presenting difficulties for the immune system.

To finish I wish to stress that what we have also learnt is that many molecular epidemiology parameters, such as turnover of peripheral population, complexity and even extent of diversity, differ with transmission and endemicity. This is yet another illustration of what has been stressed several times in this meeting: malaria is diverse and we must be cautions not to extrapolate too much from one type of endemicity to the other.

BREAKOUT SESSIONS: MALARIA VACCINES AND IMMUNOLOGY

Programme

1. Malaria Vaccines: Basic Research.

Chairs: Dr. Andrew Kitua, Professor Louis Miller.

Dr. Fred Kironde, Dr. Don Krogstad and Dr. Sirima Bienvenu

Panel : Louis Miller, Brian Greenwood, Michael Good, Soren Jepson, Wen Kilama.

Presentations

1. Basic Research - Steve Hoffman (15 minutes).
2. Interspecies conserved proteins of *P. falciparum* as potential vaccine candidates - Fred Kironde (10 minutes).
3. Immunogenicity and in vitro protective efficacy of novel recombinant multistage *Plasmodium falciparum* malaria candidate vaccine - Altaf A. Lal (10 minutes).
4. Natural immunity against Pfs 48/45 a gametocyte antigen vaccine candidate - Mike van der Kolke.
5. Role of immunoglobulins as binding molecules in rosetting of *P. falciparum* - Geoffrey Pasvol (10 minutes).
6. Rifins: A new family of *P. falciparum* proteins that are expressed on the surface of infected erythrocytes - Alexander J. Rowe (10 minutes).

Discussion and recommendations on lessons learnt and concrete plans for future.

2. Malaria Vaccines and Immunology

Chairs: Professor Marcel Tanner and Colonel Ripley Ballou

Rapporteurs: Dr. Ibrahim Elhassan and Dr. Francine Ntoumi

Presentations

1. RTS.S Overview (Introduction). - Ripley Ballou (5 minutes).
2. Schedule optimisation of the *P. falciparum* circumsporozoite hepatitis B-surphase antigen subunit vaccine RTS.S/SABS2. - Kent Kester (10 minutes).
3. Safety, immunogenicity and field efficacy studies of a *P. falciparum* malaria pre-erythrocytic vaccine. - Kalifa Bojang (10 minutes).
4. Safety and immunogenicity of the lyophylised RTS-S/SBAS2 malaria vaccine in a malaria- experienced adult population of West Kenya. - Jose A. Stout (10 minutes).
5. SPf66 - 1998 Tanzania Results. - Andrew Kitua, Pedro Alonso and Marcel Tanner (10 minutes).
6. SPf66 - Lessons learnt and future perspectives. - Pedro Alonso (10 minutes).
7. Malaria and concomitant measles infection. - Vivienne Tchinda.

Discussion (1 hour).

3. Malaria Vaccine Field Trials and Capacity Building

Chairs: Professor Brian Greenwood and. Dr. Pedro Alonso

Rapporteurs: Dr. Ritha Njau and Dr. Fulvio Esposito

Presentations (10 mins each)

1. Allelic diversity at the merozoite surface protein –1 and –2 locus of *P. falciparum* in isolates collected from Cameroonian children - Francine Ntoumi.
2. Effect of blood group, sickle cell trait and G6PD deficiency on mixed and sub-patent malaria - Olusegun Ademowo.
3. Functional analysis of *P. falciparum* EBA-175 Immunological population genetic and in vitro approaches - Daniel Okenu.
4. Identification of protective T-Cell epitopes in *P. yoelii* infection - Morris Makobongo.
5. Comparative IgG1/IgG3 antibody responses over time to MSP119 in two different areas of *P. falciparum* transmission - Olivier Garraud
6. Immunoepidemiological studies of humoral immune responses to *Plasmodium falciparum* antigens in an area characterised by seasonal and unstable malaria transmission in Sudan - Ibrahim Elhassan.
7. IFN-g Responses to infection - Adrian Luty.

Field Trials/Capacity strengthening: short, mid and long-term plans and recommendations.

Summary Report: Malaria Vaccines and Immunology

Introduction

Over 100 years after Ronald Ross 's discovery of the parasitic cause of malaria and its mode of transmission which involves the mosquito vector, malaria is still affecting 40% of the global population and is the most important public health problem of poor countries followed by HIV/AIDS.

Like other diseases, its control requires an interplay of effective strategies for providing cure to the sick and prevent the general population from getting sick. This interplay has been difficult to achieve in malaria because while effective curative drugs have been available for a long period, preventive strategies have not been adequate for most of the tropical poor countries. It has been difficult to stop transmission through vector control methods, a strategy highly advocated in the 50s and early 60s, because of the vastness of breeding sites, and the poverty has played an important role and is a major stumbling block.

The development of vector resistance to insecticides (one of the current arsenals in vector control) and parasite resistance to the existing cheap and affordable drugs like chloroquine makes malaria control a serious and difficult issue.

The development of an effective and affordable vaccine is therefore a matter of urgency in order to improve upon the current arsenals for malaria control.

Tremendous work has already been done in this area over the last two decades following the demonstration that attenuated irradiated sporozoites provides full protection to vaccinated individuals. Many vaccine candidates have been Identified although so far only a few have reached the stage of testing in humans.

The major challenges in the development of a malaria vaccine include

- Poor funding allocation to malaria vaccine development efforts. Malaria is a poor-country disease and the drug industry has had little interest in this field.
- The complexity of the parasite and its life cycle. It is generally accepted that the ideal vaccine should be multigenic and multistage. How to identify and combine the most potent antigens is a major challenge.
- The huge genome of the parasite. The P. falciparum genome project is expected to provide the major support in this area facilitating the identification of potent antigens and possible combinations.
- Difficulties to culture the parasite and produce in mass the attenuated sporozoite vaccines. Efforts in synthesizing the relevant peptides and the recombinant vaccine strategies are aimed at solving this problem.
- Active involvement of the countries with the problem in developing the vaccines and undertaking field trials.

The Multilateral Initiative on Malaria (MIM) and the recently Roll Back Malaria movement are strong indications that the world once again has recognised the important global problem of malaria and that only joint efforts can make a difference in this area. Both initiatives have and are strong advocates for the allocation of adequate funds for malaria control efforts and have in common the goal of strengthening the capacities of the poor affected countries in solving the problem. Strengthening links between northern institutions

and laboratories with advance knowledge and technology in vaccine development and southern institutions has been encouraged.

The status of malaria vaccine development, the challenges ahead and strategies to overcome them were discussed during the malaria vaccine sessions. Keynote presentations will be presented elsewhere [1, 2, 3].

1. Malaria Vaccines: Basic Research

Current research activities in this field of vaccine development include the search for new and potential molecules or genetic components belonging to the *P. falciparum* stages which can be developed as components of a new vaccines. It was reported that a new family of riffin genes that are specific to later - ring and trophozoite stages of *P. falciparum* have been identified (Rowe A. *et al*). Metabolic label experiments showed that indeed they originated from the parasite while surface labelling and trypsinazation techniques demonstrated that they are located on the surface of the parasite cell. Although their significance is not known, this family of genes is highly repeated in the genome and has potentials for vaccine development.

Promising results of a new vaccine candidate, a yiest-expressed recombinant vaccine RTS,S which contains the repeat sequences of Cercumsporozoite Surface Protein, a T-epitope and S antigen of Hepatis B were presented (Ballou R. *et al*). The vaccine formulation has been shown to be safe, immunogenic and was able to present malaria in 6/7 naive volunteers challenged with homologous parasite strains. This is a very promising vaccine candidate and currently field studies are under preparation in the Gambia and Kenya.

Another novel recombinat multistage *Plasmodium falciparum* candidate vaccine formulation termed CDC/NIIMALVAC-1 has been developed (Altaf A.Lal et al). The product is expressed in Baculovirus from a synthetic gene representing epitopes from nine *Plasmodium falciparum* antigens. Immunization in mice and rabbits elicited protective antibody and cellular immune responses to the vaccine and partner pepticle.

In the area of transmission blocking vaccines, the development of cellular immunity against Pfs 48/45 and the longevity of anti-Pfs 48/45 antibody reactivity was described (Van der Kolke et al.). Cellular and antibody immunity against Pfs 48/45 were analysed in Yaounde volunteers representing uninfected, and carrier of asexual and gametocyte stages of *Plasmodium falciparum*. *O*nly a minority of individuals exposed to gametocyte showed Pfs 48/45 dependent lymphocytes proliferation. Gametocyte careers and non-careers produced anti-Pfs 48/45 antibodies.

Rosetting of P.falciparum infected red blood cells is a phenomenon which is linked with the sequestration of P.falciparum infected red blood cells. The question is what serum components are involved in rosetting? Dr Paslvol described the role of immunoglobin in the rosetting of *Plasmodium falciparum* infected erythrocytes. In the search to assess the requirement of IgG and IgM in rosetting, an assay system was used where when schizonts were stripped of serum components and incubated in a serum free medium(Albumax I) rosetting did not occur, but was restored by additng serum to this media. It was shown that IgG depletion had no effect on the rosetting rate while IgM-depleted serum supported rosetting to only 50% of the controls and addition of purified IgM fraction increased the rosetting rate to

80% and rosette size. It was suggested that IgM is not singly involved in rosetting but throgh a complex system that may require other serum components.

Novel approaches for identifying new vaccine candidates are needed and in this respect three improved approaches for the discovery of new targets of vaccines and drugs were discussed (Kironde F, et al.). The method involves the production of anti-P.yoelii serum that can be used to probe for new interspecies conserved antigens of *Plasmodium falciparum* . In this study, by co-probing P.falciparum expression libraries with mouse anti-P.yoelii sera and rabbit anti-IMP serum, putative aepical merozoite antigen 70Kda (pf70) , an immunogenic antigen shared between P.falciparum and P.yoelii was identified and is thought to be a transmembrane molecule. A third antiserum probe specific to apical organelles of *Plasmodium falciparum* was also described.

[1,2,3] Keynote presentations by Dr. O. Puijalon, Dr. P. Hoffman and Prof. W. Kilama

2: Malaria Vaccines and Immunology

The past decade has witnessed remarkable progress in the field of malaria vaccine development. Two candidate malaria vaccines in particular (RTS,S and SPf66) have undergone intensive clinical development, including clinical trials in Africa. Our Progress in the developments of the two vaccines candidates is presented below.

SmithKline Beecham's recombinant RTS,S malaria vaccine has been under development since the late 1980s. The program has been driven by the need to establish a robust industrial manufacturing process for recombinant antigen and to identify a formulation that would induce intense but appropriate protective immune responses. The objective of the RTS,S vaccine program is to develop a vaccine that will protect children against infection with the parasite. The near term strategy is to focus on preerythrocytic antigens (CS and TRAP) and induce antibody and T cell responses that will stop the parasite before it completes liver stage development and thereby prevent blood stage infections or significantly reduce the inoculum of merozoites into the blood stream. A long term objective is to add an asexual stage antigen, such as MSP-1, to attack any few merozoites that might escape from the liver and prevent the establishment of clinically significant parasitemia. The RTS,S vaccine consists of a yeast-expressed fusion protein containing the repeat (R) region of the CS, plus its C terminal flanking region containing T-cell epitopes (T). RT is fused to the hepatitis B surface antigen (S). This RTS fusion protein is coexpressed with unfused S antigen, and spontaneously yields immunogenic particles referred to as RTS,S. A series of clinical trials revealed that a strong adjuvant was required for protection against experimental sporozoite challenge. This adjuvant is referred to as SBAS2 and is an oil-in-water emulsion containing QS21 and MPL as immunostimulants. The initial studies were performed with a liquid version of the vaccine, but it soon recognized that accelerated degradation was occurring in some clinical lots. This led to a reformulation of the antigen as a lyophilized product to which the liquid SBAS2 is added prior to injection. Results of a recent Phase I/IIa trial comparing the safety, immunogenicity and efficacy of lyophilized RTS,S compared to liquid RTS,S were presented (Kester K. *et al*). These studies confirm the safety and immunogenicity of the new formulation and reveal comparable efficacy after a two dose regimen (Å50%). Interim results of a Phase IIb field trial of liquid RTS,S in adults living in a rural Gambia community with seasonal malaria was further reported (Bojang *et al*). No efficacy data are yet available. Dr. J. Stoute reported interim data from a Phase I clinical trial of the newly lyophilized formulation in adult Kenyans living in an area of intense year-round transmission near Kisumu was also described (Stoute J. et al.). The vaccine was safe and immunogenic after first dose. Field site development in anticipation of a Phase IIb trial of the new RTS,S/TRAP vaccine later this year is in preparation. This combination vaccine has been found to be safe and immunogenic in Belgian adults, and will undergo Phase I/IIa challenge studies in the US soon.

SPf66 in an alum-adjuvanted sysnthetic peptide polymer vaccine directed against the asexual stage of the parasite that has been studied extensively over the past nine years. The vaccine was developed empirically in an academic setting and has never had the benefit of significant industrial involvement. Consequently, the product underwent limited process development before clinical trials were initiated, and this has led to many questions concerning product characterization and lot-to-lot consistency that have complicated the interpretation of the conflicting results obtained from several large field trials. Drs. M. Tanner, D. Schellenberg, P. Alonso and A. Kitua revealed publicly for the first time, the results of the most recent field

trial to have been completed with SPf66. This was a large RPCDB study involving more than 1000 infants followed at the Ifakara field site in Tanzania. The study was a follow-on study to an earlier trial carried out in Ifakara in children under 5 that indicated that the vaccine efficacy was Å31% (C1 0-52). In the new trial, the vaccine was administered to infants in a regimen that was integrated into the local EPI program. Infants were followed closely for the development of clinical malaria. The vaccine was safe and well tolerated, but no protection was observed. It was concluded that the presentation with a recommendation that no further clinical studies of SPf66 in its current formulation in Africa were indicated, but urged that the substantial investment in field site development in Ifakara be sustained.

This session was concluded by an interesting study conducted in Cameroon which investigated the relationship between measles and malaria infection and diseases (Tchinda et al). Children presenting with measles were studied by PCR to detect coinfection with malaria, and outcomes were compared. The data indicated that concomitant infection nearly doubled the mortality rate from measles in this population. If confirmed and extended to other diseases, they suggest that the beneficial effects of a malaria vaccine might extend to other important childhood illness.

Implications for Control Programs

Remarkable progress has been made and exciting candidates are being developed. However, resources are very limiting, and a licensed vaccine is at least 10 years away. Therefore, existing control programs must be strengthened and sustained. Communication between the vaccine community and control programms is essential.

Strengthening Constructive Links

The experiences in Tanzania, The Gambia and Kenya emphasize the fact that field sites for vaccine trials require sustained support, and once established, these links require continued investment. The malaria community must capitalize on capacities already developed find new ways of sharing and extending their experience and resources. We must develop a greater understanding of the epidemiology of the disease in new and existing field sites, and a recognition of the needs and expectations of the study community.

Research Capacity Needs

The vaccine community must develop the capacity to conduct more trials. Several difficult questions were raised: Are there new ways of approaching vaccine trials that would make them faster, cheaper, simpler? The notion that a malaria vaccine would need to be given with EPI may not be based on sound epidemiological data. If needed, how would one apply a vaccine outside EPI?

Resources are limited - when should development of a particular vaccine candidate stop? Can surrogate markers or correlates of immunity be used to make this process more rational?

3. Malaria Vaccine Field Trials and Capacity Building

Field trials and related activities were presented as a demonstration of additional capacities being built in the field of Malaria Vaccines.

Allelic diversity is an area which has attracted research attention in attempting to pin out the determinants of disease presentation (severe or mild). Allelic diversity at MSP-1 and 2 locus

of *P.falciparum* from a total of 73 children from Dienga, Gabon and Pooma, Cameroon were studied (Ntoumi F. et al.). Out of these 19 were symptomatic and 54 non symptomatic parasite carriers. It was shown that there was no difference in the distribution of alleles between the two groups. However carriage of allele a and b was highly anociated with the disease (a = FC 27/390 bp, b = FC 27/610 bp). There was large polymorphism with MSP-1 and MSP-2 in both places.

The question of cross sectional vs longitudinal studies in children for further exploration of this issue was raised and it was recommended that obtaining isolates from a cohort followed up over time could give more useful information.

A longitudinal study on the function of *P. falciparum* EBA-175 in terms of Immunology, population genetics and in vitro approaches was presented (Okenu et al). It involved 284 Gambian children aged from 2-9 years. Sera was collected in May (prior to transmission season) while clinical and parasitological follow-up was continued till October in order to capture malaria outcome.

The results showed that:
1. There was a strong correlation between serum reactivity of Gambian donors to the Fseg & Cseg (r = 0.85).
2. Ab prevalence to Fseg, Cseg & region III-V were age-dependent, as expected, reaching peak at adolescence.
3. Logistic regression analysis against Ab to Fseg, Cseg & region III-V did not yield evidence for protection against clinical malaria.

On the identification of protective T-cell epitopes in *P.yoelii* infection, Dr. Morris Makobango presented an interesting study which used experiments with B-cell knockout (KO) mice.

Results showed that there was:
1 Significant delayed onset of parasitaemia in immunized B-cell KO mice.
2. B-cell KO mice that received P. yoelii specific T-cells showed very low levels parasitamia (<10%).
3. (7-18K Da) soluble proteins are apparently responsible for the T-cell mediated protection.

In comparing IgG1/IgG3 Ab responses to MSP-$1_{19,}$ a study which examined the frequency, intensity and evolution over time of IgG1/IgG3 antibodies before and after the highest transmission period in groups of clinically immune adults from Dielmo and Ndiop, Senegal was presented (Garraund O. et al.). The study involved 60 individuals from each locality.

Results showed that:
1. Frequency and intensity of IgG1/IgG3 responses were significantly higher in Ndiop than in Dielmo, reciprocally to the degree of parasite exposure.
2. The data suggest that anti-MSP-1, IgG1 and IgG3 are differently, regulated after the HTP unlike before this period in clinically immune adults.
3. The importance of the dynamics in the production and utilization of IgG1 vs IgG3 in the maintenance of acquired immunity to MSP-1.

A study from Daraweesh, Sudan to determine humoral immune responses to Pf MSP-1, Pf155/RESA, CSP and GLURP in plasma sample (Elhassan I et al) showed that:

1. Antibody levels to both RESA and GLURP are increased markedly between the beginning and the end of the transmission season.
2. High titers were observed in acute plasma samples.
3. Responses to the C terminal of MSP-1 Ag occured in the majority of the acutely infected individuals.

Finally the results of a study cohort of 100 Gabonese children with either severe mild malaria (matched) was presented (Luty ATF et al). The study aim was to explore the association of IFN.R responses with resistance to reinfection with P. falciparum in young African children.

The results showed:

1. Those with severe malaria had significant shorter delay to first reinfection as well as significant higher rate of reinfections.
2. Time to first reinfection: mild = 43 weeks, severe = 29 weeks.
3. Delay to first reinfection was significant longer in individuals whose cells produced IFN-R in response to peptides derived from LSA-1 or from MSA-2 = but this association was found in the group with mild disease.

Group discussions and recommendations:

Partnership and Capacity Building

It was recognised that there were only a few centres in Africa capable of using available results of Northern Laboratories in all field in the process of developing field control tools. Hence

1. Need to strengthen existing Labs/Institutions in Africa to undertake more work on Vector Biology, Molecular Epidemiology, Pathogenesis, Immunology and Molecular Parasitology.
2. Establish and maintain capacities for *Plasmodium falciparum* culture in order to test new drugs. Regional Labs with this capacity could act as supplies of *Plasmodium falciparum* culture materials to other labs as need arises.
3. Establish at least on regional basis centre/labs capable of conducting vaccine challenge trials establishing protection in semi-vaccine immune populations.
4. Increase the number of sites capable on conducting Phase I-III Vaccine trials.
5. The donor community must be sensitized and advocacy for malaria control which required an efficacious vaccine/s should be maintained. Malaria elimination will make the world a lot better and healthier place to live in.

Research Priorities

Short Term:

1. Complete development of new candidates (RTS,S & others) should be accelerated.
2. Consideration of the impact of parasite diversity on vaccine design is essential.
3. Identification of better endpoints and surrogate markers to make trials easier, faster, less expensive should also be accelerated.
4. There is need for in-depth study for vaccine candidates in field conditions.
5. Suitable models to predict what happens on humans are required.
6. Available genotype data should be analysed in order to see what they present in the large scale.

7. On the choice between polymrphic versus conserved antigens for vaccines, it was suggested that conserved genes are preferable.

Medium Term:

1. Identification of an effective vaccine remains the goal
2. Pivotal Phase III trials in target populations must be designed and executed to high standards
3. There is need for longitudinal studies to determine the turnover rates for some genotypes and their significance in relation to clinical presentation.
4. In depth studies on Var-genes and the role of gametolyte carriers are needed.
5. There is need to monitor the response of MUST DO 15 and extend the current findings as well as sorting out the components of MUST DO 5.

Long Term:

1. Consider how best to implement a vaccine into control programs
2. Evaluate the impact of vaccination in early life.

Link Between Research and Control

1. As yet no vaccine is available for control and therefore the message to the control community is that while waiting for the vaccine, utilization of existing tools in synergistic manner can make a difference.

2. Insecticide impregnated mosquito nets have been shown to reduce overall mortality of children by 35% and their continued use together with improvement in early diagnosis and proper case management will save many lives.

3. Communities must be encouraged and helped by all means to protect themselves from mosquito bites, clean the environment and reduce mosquito breeding sites and use prompt diagnosis and treatment as part of their culture for health living.

4. For effective malaria control, efforts must be directed at the development of an efficacious vaccine within the next ten years.

5. For Africa, the best choice is a vaccine that would reduce mortality. An ideal vaccine would be multigenic and multistage and efforts to produce such a vaccine, which would be able to counteract all stages of the parasite are being made.